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MICROBIAL COMMUNITY DIVERSITY INFLUENCED BY ORGANIC CARBON SOURCES IN RICE - RAPE ROTATION FARMLAND

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Abstract

The present study was intended to characterize the responses of soil microbial community to organic carbon sources in rice-rape rotation farmland in Erhai Basin, Yunnan, China. The farmland was supplemented with nitrogen fertilizer alone, nitrogen fertilizer plus organic carbon source materials (Vicia faba straw, corn straw, or composting pine needles) or not fertilized; the soil physical and chemical properties, carbon and nitrogen hydrolases (β - 1,4-glucosidase, N-acetyl - β - D-glucosidase) were quantitatively investigated. The composition and diversity of soil microbial community were scrutinized by Illumina high-throughput sequencing technology. The effects of soil physicochemical properties on soil microbial community were revealed by redundancy analysis (RDA). It turned out that when compared with no fertilization, the organic fertilizers plus nitrogen fertilizer significantly increased organic matter, total nitrogen, carbon to nitrogen ratio (C / N ratio) in soils and the activities of carbon/nitrogen hydrolases by 7%, 3%, 5% and 22%/9%, respectively. The results of high-throughput sequencing showed that organic carbon source materials combined with nitrogen fertilizer significantly altered the composition and diversity of soil microbial community; *Actinoplanes*, *Rhizobiales*, *Sordariales*, *Chaetomiaceae* and *Labilithrix*, which were able to degrade organic matter and fix nitrogen, were substantially increased in their relative abundance. RDA showed that the rhizosphere microbial community was significantly affected by pH, organic matter, total nitrogen and C / N ratio (p<0.05), and *Labilithrix*, *Sordariales*, *Actinoplanes*, and *Chaetomiaceae* showed a significantly positive correlation with total nitrogen, organic matter and C / N ratio. This study indicates that organic carbon source materials combined with nitrogen fertilizer could increase the contents of soil organic matter and total nitrogen, enhance the activities of carbon/nitrogen hydrolases, and significantly increase the relative abundance of microorganisms capable of degrading organic matter and promoting nitrogen cycle in soils.

Key words: Fertilization treatment; Soil microbial community; High-throughput sequencing; Carbon and Nitrogen Coordination effect; Redundancy analysis.

Introduction

Modern agriculture was confronted with the problem of low utilization rate of chemical fertilizer, which causes soil and water eutrophication. In the Erhai Lake Basin of Yunnan Province, southwest China, the fertilizer utilization rate of farmland is only 22% - 40%, and the surplus nitrogen (N) flows into Erhai Lake to pollute water sources and lakes (Shi & Xu, 2013). As a tributary of the Lancang River, Erhai Lake Basin mainly adopts rape-rice rotation mode (Fig. 1), and the main soil types were red soil, paddy soil and limestone soil. Huang T *et al*., (2013). Put forward the fertilization technology mode of combining organic and inorganic fertilizers to maximize carbon and nitrogen management in Erhai Lake Basin.

Lei *et al*., (2014) showed that carbon and nitrogen in soil had functional interaction (e.g., cooperation, dependence and transformation). Therefore, carbon and nitrogen coordination effect suggest that the application of organic carbon source could effectively improve the utilization efficiency of soil nitrogen. Soil microbial community is an essential part of the ecological cycle system in the soil rhizosphere region (Zhou *et al*., 2020). The differential richness of different communities could closely affect the use of carbon and nitrogen by plants, while soil pH, organic carbon and nitrogen content will also significantly affect the microbial community construction (Porporato *et al*., 2004; Masoud *et al*., 2019; Cai *et al*., 2020; Vanina *et al*., 2020). High carbon storage in the soil was conducive to the absorption of soil amino acids and trace elements by crops, and could also promote the accumulation of secondary metabolites of microorganisms (Chen *et al*., 2020; Pan *et al*., 2020; Ye *et al*., 2020). The effects of exogenous organic carbon sources and nitrogen fertilizer on the diversity of rhizosphere microbial communities have become a fierce and tough topic in recent years. Diverse microbial communities display different functions. Presently, Illumina high-throughput sequencing technology is frequently used to determine the richness of the soil microbial communities (Maia *et al*., 2019; Zhang *et al*., 2020). Through the determination of microbial community and functional potentials, it was found that as the main organisms of carbon and nitrogen transformation in soil (Koshlaf *et al*., 2020) microbes can transform the carbon and nitrogen components, which are difficult to be used by plants, into compounds that were easy to be absorbed by plants. Therefore, the quantification of soil microbial community can provide a theoretical basis for the study of carbon nitrogen interaction.

Fig. 1. Crops and their habitats in Erhai Lake Basin of Yunnan Province, China. A, Rape; B, Rice; C, Rhizosphere soil; D, Plot; E, Fertilizer, fertilizer + organic materials.

Liu C *et al*., (2019) carried out the research on the relationship between crop growth and soil microbial communities, and found that the increase of carbon content was conducive to crop growth and the accumulation of microbial metabolites. Wang *et al*., (2020) examined the abundance of soil microbial community after straw and carbonized straw treatment, and found that the content of soil organic matter increased significantly after amendment with straw and carbonized straw (p<0.05); in the metronymic study, it was found that the Actinobacteria and Bacteroidetes in soil increased significantly in relative abundance after straw returning to field directly (p<0.05). The carbonized straw dramatically increased the relative abundance of *Acidobacteria*. Democrat *et al*., (2019) found that there was a competitive relationship among soil microbial communities. It was also found that the degree of soil drought and the type of vegetation cover would cause changes in soil properties, and significantly affects the composition and diversity of soil microbial communities. The input of organic carbon and nitrogen sources significantly increased the content of soil Proteobacteria in the bacterial community (Berg & Smalla, 2009). Ammonia oxidizing bacteria (AOB), which has a role in nitrogen fixation, could transform soil nitrogen into nitrite and improve crop absorption (Zhao *et al*., 2017). In the fungal community, the abundance of saprophytes that degrade organic matter and cellulose was also increased substantially by straw returning (Dai *et al*., 2019). However, few studies have examined how the rhizosphere microbial community alters in the context of high carbon storage in paddy field environment. We hypothesized that the rhizosphere microbial community diversity could be profoundly influenced by the addition of organic carbon sources in rice - rape rotation farmland.

In the present study, different treatments were conducted in the experimental field, e.g., sole application of chemical fertilizer (T1) and organic fertilizer (corn straw T2, Vicia faba straw T3, composting pine needle T4) combined with nitrogen fertilizer. The amendment lasted for seven years. The changes in soil pH, organic matter, total nitrogen content and the soil microbial community were monitored and measured, and the relationship between different treatments was summarized through experimental data. It turned out that under the high carbon background, the intake of organic carbon and nitrogen sources could significantly alter the soil microbial community in rice - rape rotation farmland, resulting in the enhancement of carbon source degradation and nitrogen fixation in soil of Erhai Basin, Yunnan, China.

Materials and Methods

Overview of the study area and experiment design: The experimental field was located in Erhai Basin (98°52’- 101°03’ E, 24°41’ - 26°42’ N), Dali City, Yunnan Province. The altitude was 2,056 m and the annual average temperature 15.1°C and the average rainfall 838 mm and the highest temperature in August (18°C - 28°C), and the lowest temperature in December (3°C - 13°C), which belongs to the typical subtropical monsoon climate.

In order to eliminate the interference of environmental heterogeneity, the same rice - rape rotation farmland of 30 m^2 was selected as the experimental area. The control and different experimental treatments were set up respectively in a total of 24 plots with the interval of 1 m. With no organic carbon source material and chemical fertilizer as control (CK), five experimental treatments were set up, including chemical fertilizer alone (T1), corn straw combined with chemical fertilizer (T2),

broad bean straw with chemical fertilizer (T3), and composting (decomposed) pine needles plus chemical fertilizer (T4); there were three replicates for each treatment. The experimental processing was highlighted for fixed-point and timing detection. Nitrogen fertilizer was urea with 46% N (mass fraction). Phosphate fertilizer was calcium superphosphate with 16% P_2O_5 and the potassium fertilizer was potassium sulfate with 50% K_2O. The application rate of organic materials and chemical fertilizer was 1,250kg / hm^2, and the treatment duration until this study was seven years.

Sample collection and determination: The sampling time was on June 2017 and the location was the experimental area of Erhai Lake Basin. Soil samples were collected during the harvest period of rape (Brassica nap). The 5-point sampling method was used to drill 0-20cm soil surface layer. Five samples were drawn from each plot to make a composite. The soil samples were stored in a 50 ml centrifuge tubes, put into an ice bag and brought back to the laboratory for storage at – 20°C. The main physical and chemical indexes were determined by experimental analysis, the enzyme activities were determined by the colorimetric method with spectrophotometer, and soil samples were sent to Shanghai Personal Biotechnology Co., Ltd. for high-throughput amplicon sequencing of microbial community.

Determination of soil physicochemical properties: After removing impurities, the collected fresh soil samples were dried with a freeze dryer, and then ground and filtered through a 1mm sieve. The soil was combined with sterile water in the ratio of 1:5 (g / mL), and the pH value was measured by CS desktop pH meter; the nitrogen content was determined by the flow analyzer. One mol / L K_2SO_4 solution was added into the soil to extract for 2 h, and then the organic matter was quantified (Zhang *et al*., 2019).

Determination of enzyme activity by spectro-photometer: The soil N-acetyl - β - D-glucosidase (NAG) activity and β - 1,4-glucosidase (GC) activity were determined by DNS colorimetry (Wu *et al*., 2014; Nong *et al*., 2019) using 2g and 3g soil samples, respectively. The incubated tube was centrifuged at 4°C for 10 min at 12,000 rpm. The spectrophotometer was preheated for more than 30 min to reduce the test error and the wavelength was adjusted to 540nm. A total of 200 μL supernatant of the treatment samples were transferred respectively to the 96 well plate, and the absorbance value was determined at 405 nm (Qazi *et al*., 2014; Wang *et al*., 2019).

Extraction of soil microbial DNA and high throughput amplicon sequencing: Power Soil DNA Isolation Kit (MoBio, US) was used to extract microbial DNA from soil. The V3-V4 region of bacteria 16S rRNA (PCR primers 338F 5'- ACTCCTACGGGAGGCAGCAG -3' and 860R 5'- GGACTACHVGGGTWCTAAT -3') and fungal ITS1 (ITS1F 5'- CTTGGTCATTTAGAGGAAGTAA -3' and 2043R 5'- GCTGCGTTCTTCATCGATGC -3') were PCR amplified (Ellington *et al*., 2016; Zhao *et al*., 2019). The 25.0 μL PCR reaction mixture for 16S rRNA was composed of 1 μL genomic DNA template, 2.5 μL Mg^{2+} free buffers, 1.5 μL Mg^{2+}, 2.0 μL dNTP, 1 μL forward primer, 1 μL reverse primer, 0.2 μL Taq DNA polymerase, and deionized water 15.8 μL. The PCR condition was: 95°C denaturation for 2 min; 50 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 30 s; 72°C for 5 min. The 20 μL PCR reaction mixture for ITS was composed of 10 ng genomic DNA template, 4.0 μL 5× FastPfu buffer, 2.0 μL 2.5 mmol/L dNTP, 0.8 μL 5 μmol/L forward primer, 0.8 μL 5 μmol/L reverse primer, 0.4 μL FastPfu polymerase, and deionized water. The PCR condition was: 95 ° C denaturation for 3 min; 50 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 30 s; 72°C for 10 min.

The PCR amplified products were tested and purified, and the sequencing library was constructed. The high throughput amplicon sequencing was performed as previously reported (Hao *et al*., 2016).

Data analysis

The bioinformatic analysis was performed as previously reported (Hao *et al*., 2016). Alpha diversity indices Chao1, Simpson and Shannon were calculated (http://scikit-bio.org/docs/latest/generated/generated/skbio. diversity. alpha. chao1 and Shannon and Simpson. Html). LEfSe (LDA Effect Size) was performed via Galaxy (http://huttenhower. sph. harvard. edu/galaxy/); the relative abundance matrix of the genus level was submitted for LEfSe analysis. The composition of each classification level has been automatically analyzed by LEfSe, and the analysis results have been visualized. Since there were many environmental factors affecting the sample composition, Variance Inflation Factor (VIF) analysis was performed to screen the influencing factors before redundancy analysis (RDA), Pi = 1 / (1-Ri2), where Ri was an independent variable; the larger the value was, the more serious the multicollinearity between independent variables was. It has been set to 3.5. The relationship between microbial population and environmental factors can be effectively analyzed by RDA, after the removal of multicollinearity.

Results

Effects of fertilization treatments on pH, C / N ratio and organic matter in rhizosphere soil: The physical and chemical properties of soil samples were first determined. There were significant differences in soil pH, C/N ratio and organic matter content (p<0.05) between different treatments, i.e., no fertilization (CK), chemical fertilizer (T1), and organic fertilizer (composting pine needle T2, broad bean straw T3, corn straw T4) plus chemical fertilizer. As shown in Table 1, there was no significant difference in total nitrogen ($g \cdot kg^{-1}$) and C / N ratio between CK and T1 (p>0.05), while in organic fertilizer-applied treatment (T2, T3, T4 mean value), organic matter ($g \cdot kg^{-1}$), total nitrogen ($g \cdot kg^{-1}$) and C / N ratio were significantly increased (p<0.05) by 7.1%, 3.3% and 5.0% respectively, as compared with T1.

Table 1. Effects of carbon sources and chemical fertilizers on soil pH, organic matter, total nitrogen and carbon nitrogen ratio.

Treatment	pH	Organic matter (OM, g·kg^{-1})	Total nitrogen (TN, g·kg^{-1})	C/N ratio
CK	5.52 (0.08)	42.76 (1.00)	2.41 (0.01)	10.28 (0.33)
T1	5.25 (0.04)	41.95 (1.29)	2.46 (0.04)	9.88 (0.25)
T2	5.34 (0.03)	46.83 (3.19)	2.57 (0.01)	10.60 (1.20)
T3	5.36 (0.06)	47.78 (2.14)	2.57 (0.01)	10.77 (0.86)
T4	5.07 (0.49)	49.64 (1.16)	2.58 (0.01)	11.16 (0.37)

Note: The data in the table are the average value (standard deviation) of soil samples. The pH under five conditions was not significantly different (p = 0.07). The organic matter and total nitrogen under T2-T4 treatments were significantly higher than those under CK and T1 (p = 0.04, ANOVA). The C/N ratio under T4 was significantly higher than that under other conditions (p = 0.03), and the C/N ratio under T2 and T3 was significantly higher than that under CK and T1 (p = 0.02)

Table 2. Alpha diversity analysis of soil microbial community.

	Item	CK	T1	T2	T3	T4
16S rRNA	Simpson index	0.003 (0.0001) ^{ab}	0.002 (0.0006) ^{b}	0.002 (0.0002) ^{b}	0.003 (0.0006) ^{ab}	0.004 (0.0003) ^{c}
	Shannon index	6.92 (0.10) ^{a}	7.02 (0.12) ^{a}	7.03 (0.07) ^{a}	6.96 (0.06) ^{a}	6.89 (0.05) ^{a}
	Chao1	4025.58 (367.5) ^{a}	4129.37 (173.61) ^{a}	4305.21 (98.38) ^{a}	4252.54 (28.39) ^{a}	4171.43 (187.2) ^{a}
ITS	Simpson index	0.11 (0.1) ^{ab}	0.07 (0.02) ^{bc}	0.19 (0.05) ^{a}	0.06 (0.01) ^{b}	0.16 (0.03) ^{a}
	Shannon index	3.35 (0.91) ^{bc}	3.68 (0.13) ^{b}	2.62 (0.42) ^{c}	3.81 (0.08) ^{bc}	2.83 (0.4) ^{a}
	Chao1	579.5 (126.21) ^{a}	533.41 (37.48) ^{a}	484.03 (82.35) ^{a}	530.43 (45.6) ^{a}	508.32 (162.64) ^{a}

The data was the mean value (standard deviation) of 3 repeats. Different lowercase letters indicated the difference of significance at the level of 0.05, the same was below

Changes of soil carbon/nitrogen hydrolase activity and influencing factors: The results showed that the activities of soil hydrolases (NAG and GC) were significantly different under different treatments (p<0.01). As shown in Fig. 2A, the activities of NAG and GC in T2 group increased by 9% and 22% respectively; in T3 group, they increased by 8% and 23% respectively, and in T4 group, they increased by 9% and 21% respectively, as compared with those of chemical fertilizer alone (T1). The activity of nitrogen hydrolase (NAG) in T1 increased by 13%, as compared with that of CK. RDA (Fig. 2B) showed that the resolution of axis 1 (x axis) was 87%, and the environmental factors that significantly affected carbon/nitrogen hydrolases were organic matter and total nitrogen content, which were positively correlated with the hydrolase activities. Additionally, the effect of organic matter on GC could be greater than that on NAG.

In the bacterial community (Table 2), the Shannon index under five conditions was not significantly different, so were the Chao1 index and Simpson index (p>0.05). In the fungal community, the Shannon index was significantly higher (p<0.05) and Simpson index was significantly lower (p<0.05) in T4 than in T1.

In this study, the UniFrac beta diversity of five communities was analysed, and the similarity and difference among the samples were evaluated by visualized using principal coordinate analysis (PCoA). As for the bacterial community, the contribution rate of the first and second principal coordinates in UniFrac PCoA (Fig. 3A) were 23.9% and 11.4% respectively, and they accumulatively explained 35.3% of total variance. In the fungal community (Fig. 3B), the first and second principal coordinates explained 35.4% and 16.0% of total variance, respectively. Based on the abundance difference at the OTU level under different treatments, the sample groups were well separate from each other.

Analysis of species with significant difference: According to the linear discriminant analysis (LDA) (Fig. 4) of rhizosphere microbiota, the *Chloroflexi* group KD4_96 had the LDA value of 4.09 under control treatment, which was greater than the default value 2.0, suggesting that the relative abundance of this taxonomic group was significantly higher in CK than in other treatment groups. The sole application of nitrogen fertilizer (T1) substantially enriched the Proteobacteria genus *Cupriavidus*; under T2, the LDA value of Proteobacteria genus *Acidibacter* was 2.85, and the LDA values of *Actinoplanes* and *Rhizomicrobium* were also greater than the default value 2.0, suggesting that the relative abundance of these three genera was significantly higher in T2 than in other treatments. In addition, the biomarker genera of T3 were *Labilithrix*, while T4 significantly enriched the genera *Alterococcus* and *Phenylobacterium* (Fig. 4A). In fungal communities (Fig. 4B), the LDA value of *Nectriaceae* was 5.09 in CK, the LDA value of *Bionectriaceae* was 4.86 under T1 treatment, and other enriched taxonomic groups could also be the biomarker in the respective treatment group. The addition of organic fertilizers also enriched *Sordariales* and *Chaetomiaceae* in T2 and T3 respectively, while *Sordariomycetes* and *Teratosphaeriaceae* differentiated corn straw amendment (T4) from other treatments. In a word, organic fertilizers differentially increased the diversity and richness of soil microbial community, and the biomarker groups well represent the differences of microbial community groups under different treatments.

Functional analysis of core microbiome: The above analysis found a large number of unclassified microorganisms in CK, and the biomarker groups in different treatments included α-Proteobacteria, β-Proteobacteria, Actinobacteria, and the fungi *Chaetomiaceae* and *Sordariales*, etc. Their functions were shown in Tables 3 and 4. For example, *Nectriaceae* exists in animal and plant matrices in the form of parasitism or saprophytic fungi, and promotes the transformation of soil humus and the growth of plant seedlings. *Bennettitaceae* could maintain ecological balance and transform metabolites. Among the bacteria with differential abundance under different treatments, *Chloroflexi* could oxidize and reduce nitrite in soil (Garrido-Oter *et al*., 2018). Aerobacter can promote nitrification of nitrogen in soil (Wang Chiung-Mei *et al*., 2019), which was conducive to the accumulation of nitrogen in soil.

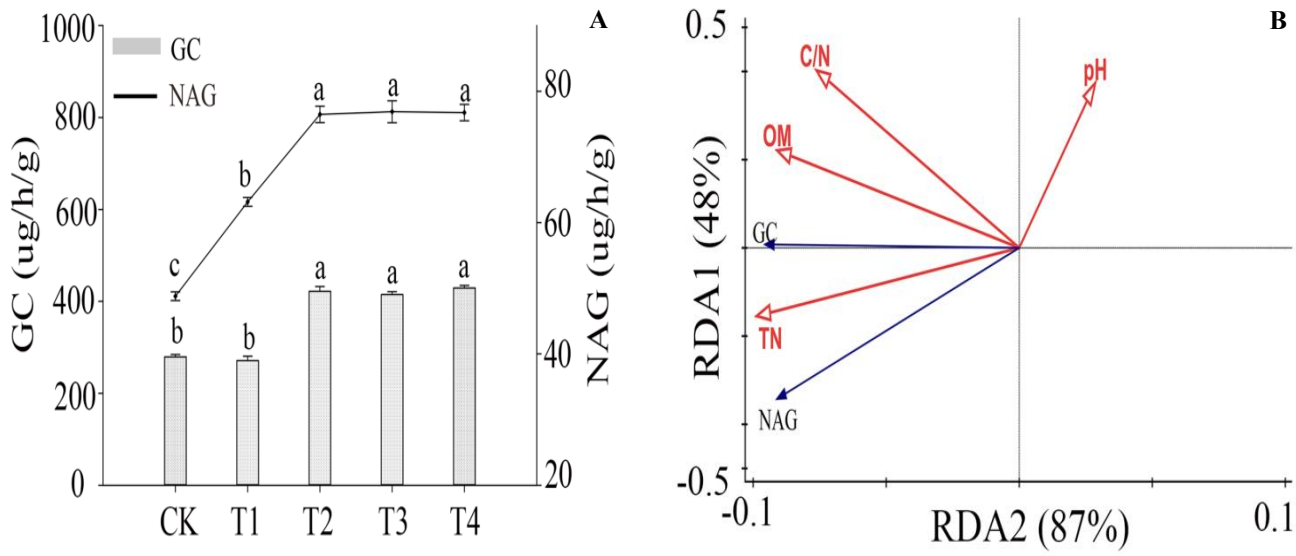

Fig. 2. Effects of different carbon and nitrogen sources on sngth of 240-490 bp. At the similarity level of 97%, 1,470 OTUs were inferred.

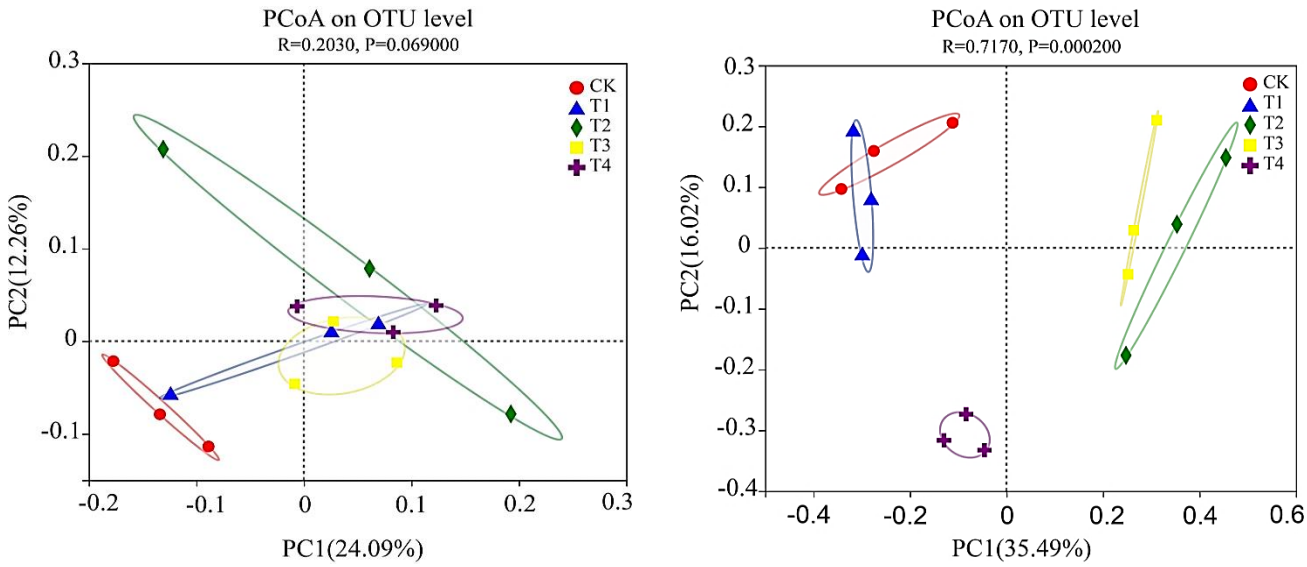

Fig. 3. PCoA analysis of rhizosphere microbiota under different treatments.

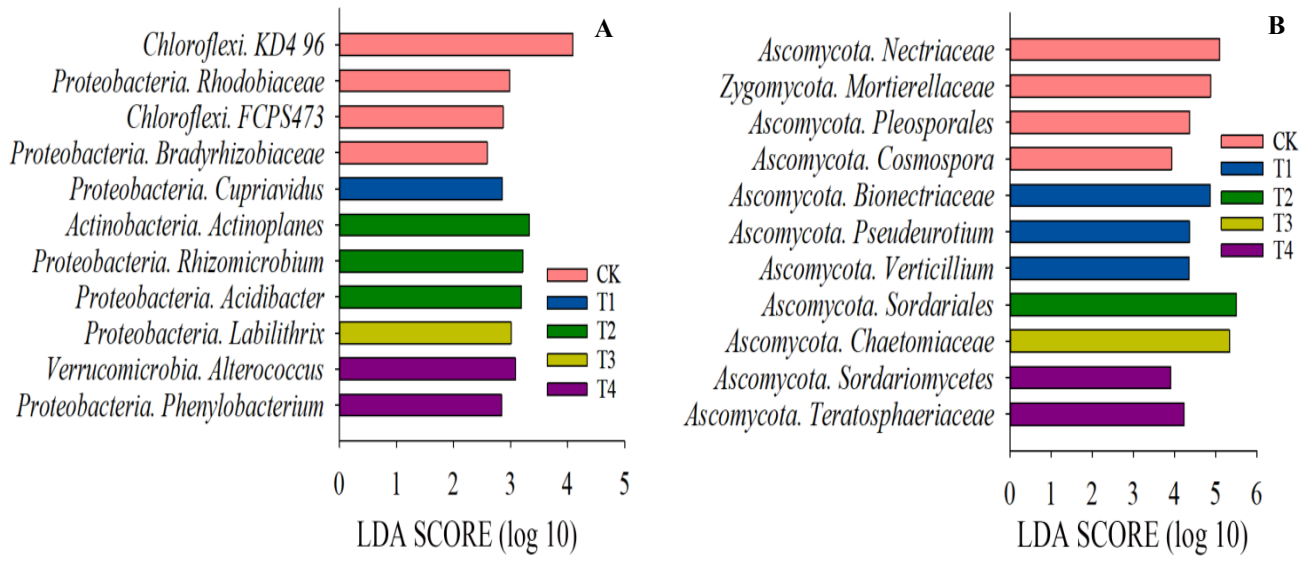

Fig. 4. LDA of rhizosphere microbiota a under different treatments.

The addition of organic carbon source could effectively change the abundance of the soil microbial community, and its function alters accordingly. Saprophytic fungi *Sordariales* and *Chaetomiaceae* were conducive to the decomposition of soil humus and the nitrogen fixation (Liu *et al*., 2013; Dong *et al*., 2018) (Table 3). Rhizobia had the function of nitrogen fixation (Yu *et al*., 2020), and *Verrucomicrobia* can degrade cellulose in soil and promote the decomposition of organic materials (Awal *et al*., 2016) (Table 4). Therefore, organic fertilizers promoted the activity of nitrogen fixation and organic matter degradation through microorganisms in rice-rape rotation farmland.

Impact factors of rhizosphere microbial community: RDA results (Fig. 5) showed the adaptability of microbial genera/families with differential abundance of edaphic conditions and the influence of soil physical and chemical factors on soil microbial community. For fungal and bacterial community, axis 1 (x axis) explained 60% and 80% of total variance, respectively, which contain most of the information of soil environment and microbial community, and can effectively explain the relationship between different physicochemical properties and samples. The soil pH, total nitrogen and organic matter had significant effects on rhizosphere bacterial community (p<0.05) (Fig. 5A). The application of organic fertilizers increased the abundance of *Actinoplanes*, *Rhizobiales*, and *Labilithrix*. Therefore, organic matter and total nitrogen were the main environmental factors causing community alteration, and there was a positive correlation between these factors and some bacterial groups. The angle between Chloroflexi.KD4.96, *Rhodobiaceae*, *Bradyrhizobiaceae* and pH was less than 90 degrees, suggesting the positive correlation between these taxonomic groups and pH. On the other hand, soil pH, total nitrogen, organic matter, C / N ratio had significant effects on the rhizosphere fungal community (p<0.05) (Fig. 5B). The total nitrogen, organic matter and C / N ratio were positively correlated with alterations of some fungal groups. The angle between *Nectriaceae*, *Mortierellaceae*, *Pleosporales* and pH was less than 90 degrees, suggesting the positive correlation between these taxonomic groups and pH.

Discussion

Effects of different carbon sources and chemical fertilizers on pH, C / N ratio, organic matter and enzyme in rhizosphere soil: As an important indicator of rhizosphere microbial activity, soil organic matter determines soil fertility (Fujiwara *et al*., 2013). Total nitrogen represents the total amount of nitrogen in soil, reflecting the level of protein and polypeptide in soil (Meidute *et al*., 2008). As shown in Table 1, the organic matter, total nitrogen and C / N ratio of soil were dramatically increased by the addition of three carbon sources (composting pine needles, broad bean straw and corn straw), as compared with those of CK, which suggest that organic fertilizers plus chemical fertilizer increased soil fertility and protein content, which may be related to the improved soil nutrients, texture and porosity by the supplementation of organic carbon.

N-acetyl - β - D-glucosidase (NAG), as a nitrogen related hydrolase in soil, plays its role under the condition of acidic pH (Li *et al*., 2015). β - Glucosidase (GC) was a kind of cellulose hydrolase, which degrades cellulose in the soil and provides energy for rhizosphere microorganisms (Meng *et al*., 2008). As showed in Fig. 2A). The application of organic carbon and nitrogen sources increased the activities of NAG and GC in soil; extraneous input of organic carbon/nitrogen could promote the cellulose decomposition in soil and accelerate the carbon cycle. RDA (Fig. 1B) showed that organic matter and total nitrogen content significantly influenced the activity of C-N hydrolase, and the application of an organic carbon source could increase the activity of C-N hydrolase.

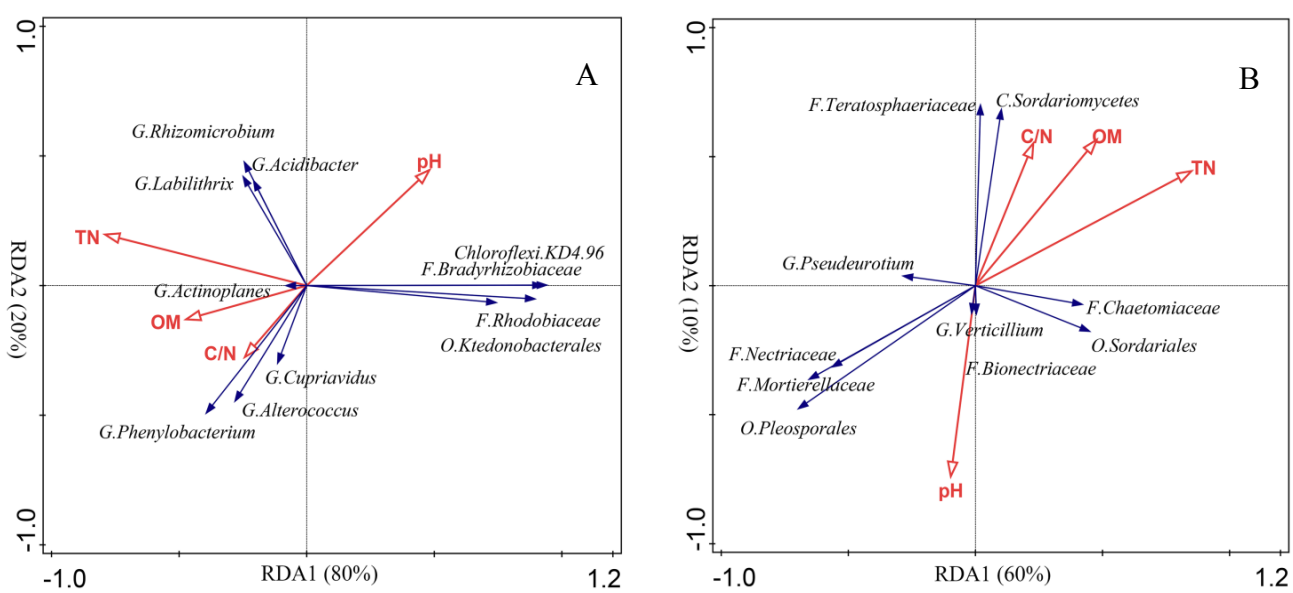

Fig. 5. RDA showing the effect of environmental factors on microbial groups with significantly different abundance under different fertilizer treatments. A, bacteria; B, fungi.

Table 3. Effects of organic and chemical fertilizers on soil fungal communities: Biomarker groups and their potential functions.

Treatment	Species name	Major function
CK	p__*Ascomycota*. *f__Nectriaceae*	It exists in the animal and plant matrix in the form of parasitism or saprophytic, and promotes the transformation of saprophytic substance (Cao *et al*., 2016)
T1	p__*Zygomycota*. *f__Mortierellaceae*	It is saprophytic in animal and plant residues, can reproduce sexually and asexually, and can cause plant disease (Zhuang, 2010)
	p__*Ascomycota*. *o__Pleosporales*	It is parasitic or saprophytic, and can degrade cellulose (Yadav *et al*., 2014)
	p__*Ascomycota*. *g__Cosmospora*	It is parasitic or saprophytic, and can promote the transformation of humus (Yuan *et al*., 2020)
	p__*Ascomycota*. *f__Bionectriaceae*	It is parasitic or saprophytic, can maintain ecological balance, and transforms metabolites (Wang *et al*., 2015)
T2	p__*Ascomycota*. f__Pseudeurotiaceae	It mainly exists in the form of parasitism or saprophytic and has antagonistic activity (Schroers, 2002)
	p__*Ascomycota*. g__Verticillium	As a biological control agent, it is used to control pests (Vanderwolf *et al*., 2016)
	p__*Ascomycota*. *Sordariales*	As the saprophytic growing in manure, soil, rotten wood, rotten plants and other basic materials, it is conducive to the transformation of nitrogen and other elements (Liu *et al*., 2013)
T3	p__*Ascomycota*. *f__Chaetomiaceae*	As the saprophytic growing in organic matter, it is conducive to the transformation of nitrogen and other elements (Dong *et al*., 2018)
T4	p__*Ascomycota*. *f__Teratosphaeriaceae*	It is favorable for organic matter transformation and can produce lipase to degrade organic matter (Dong *et al*., 2018)
	p__*Ascomycota*. *f__Sordariomycetes*	As the saprophytic growing in manure, soil, rotten wood, rotten plants and other matrices, it is conducive to the transformation of nitrogen and other elements (Dong *et al*., 2018)

Note: p, phylum; o, order; f, family; g, genus

Table 4. Effects of organic and chemical fertilizers on soil bacterial communities: Biomarker groups and their potential functions.

Treatment	Species name	Major function
CK	p__*Chloroflexi*. *c__KD4_96*	No oxygen is produced in photosynthesis, and nitrite is oxidized and reduced (Hyde *et al*., 2017)
T1	p__*Proteobacteria*. *f__Rhodobiaceae*	Facultative or obligate anaerobic and heterotrophic life, symbiosis with plants, conducive to nitrogen cycle (Wang *et al*., 2015)
	p__*Chloroflexi*. *f__FCPS473*	Nitrite is oxidized and reduced (Garrido-Oter *et al*., 2018)
	p__*Proteobacteria*. *f__Bradyrhizobiaceae*	Symbiosis with plants, with nitrogen fixation function (Wang *et al*., 2019)
	p__*Proteobacteria*. *g__Acidibacter*	Nitrification can occur, which is conducive to the transformation of nitrogen (Zhang C *et al*., 2020)
T2	p__*Proteobacteria*. *g__Cupriavidus*	Colonize in the root and rhizosphere, promote enzymatic transformation of nitrogen (Wakimoto T *et al*., 2020)
	p__*Actinobacteria*. *g__Actinoplanes*	Saprophytic bacteria play a role in the natural nitrogen cycle (Lin *et al*., 2018)
	p__*Proteobacteria*. *f__Rhizobiales*	Symbiosis with plants, with nitrogen fixation function (Yu *et al*., 2020)
T3	p__*Proteobacteria*. *g__Labilithrix*	Chemotactic heterotrophic, mesophilic and aerobic, with nitrogen fixation function (Jang *et al*., 2017)
T4	p__*Verrucomicrobia*. *g__Alterococcus*	It is parasitic and mainly grows in soil and water; it can degrade hemicellulose and participate in carbon and nitrogen cycle (Awal *et al*., 2016)
	p__*Proteobacteria*. *g__Phenylobacterium*	Symbiosis with plants, with nitrogen fixation function (Ohta *et al*., 2004)

Note: p, phylum; o, order; f, family; g, genus

Species with differential abundance and function analysis: LDA was used to find taxonomic groups that were significantly different in abundance among treatment groups (Xu J *et al.,* 2018). LDA value can be used to estimate the effect of abundance of each taxonomic group on the difference of treatment groups. For instance, LEfSe was used to analyze the bacterial community in the marine acidic sediment and find that Proteobacteria was the dominant microflora. In the present study, soil pH value was one of the main factors affecting bacterial community. We found that the addition of three organic fertilizers (T2 - T4) effectively improved the abundance of *Proteobacteria* and *Acidobacteria* in farmland, partially because that the input of organic carbon sources accelerates the metabolism of eutrophic groups, and rhizobia and *Acidobacteria* effectively transform nitrogen atoms into nitrite which is conducive to the balance of carbon/ nitrogen sources in soil. The abundance of *Chaetomiaceae* and *Sordariales* also increased significantly. These fungi could degrade cellulose and promote the transformation of organic matter in soil. In a word, the addition of organic and chemical fertilizers can lead to significant differences in microbial community structure/composition and increase the abundance of microbes that can degrade organic matter and fix nitrogen in the soil. This may be due to the fact that a large amount of organic carbon and humus enable microorganisms to efficiently use for their own growth.

Impact factors of soil microbial community: The soil pH, total nitrogen, organic matter and C / N ratio could cause changes in microbial community richness/diversity, and pH has a significant impact on soil bacterial community. When the soil was in weak acid condition, it was conducive to the growth of denitrifying bacteria, and could stimulate the growth of facultative anaerobic and aerobic bacteria capable of organic matter degradation (Chung *et al*., 2010). The application of organic carbon source (e.g., corn straw) could increase the abundance of bacteria *Dactylosporangium* and *Chloroflexi*, and fungi *Chaetomiaceae* and *Sordariales* (Kerr *et al*., 2020). In this study, LDA (Fig. 5) was used to obtain the biomarker taxonomic group with significant difference among fertilizer treatments. RDA showed that the abundances of *Actinoplanes*. *Rhizobiales*, and *Labilithrix* were sensitive to changes in total nitrogen and organic matter, and they was positively correlated with environmental variables (soil pH, organic matter, total nitrogen, C / N ratio). The fungi *Sordariales* and *Chaetomiaceae* were also sensitive to total nitrogen and organic matter. The addition of organic fertilizers in the soil increased the abundance of *Labilithrix* and *Chaetomiaceae*. These microorganisms could degrade organic matter and fix nitrogen. RDA showed that compared with other microbial groups, the angle between organic matter/total nitrogen and Labilithrix/Chaetomiaceae was the smallest, implying that these two microbes were more suitable for soil conditions. By improving the structure of the soil microbial community, it was possible to further improve the utilization rate of the nitrogen source in the soil.

Conclusion

For the first time, the responses of soil microbial community to organic carbon source materials were depicted in rice-rape rotation farmland over a span of seven years. Three organic fertilizers (corn straw T2, Vicia faba straw T3, composting pine needle T4) combined with chemical fertilizer were applicable to the farmland to investigate the alterations of rhizosphere microbial community in the context of high carbon storage in paddy field environment. The amendment went on for seven years. In the rice-rape rotation farmland in Erhai Basin of southwest China, the application of organic carbon source materials significantly increased the soil organic matter, total nitrogen. They also increased the C / N ratio and carbon/nitrogen hydrolase (GC and NAG) activities (p<0.05). The application of organic fertilizers led to the significant differences in microbial community structure and composition as compared with no fertilizer and chemical fertilizer alone; as the long-term effect, three tested organic fertilizers meaningfully increased the relative abundance of *Sordariales*, *Sordariomycetes*, *Rhizobiales* and *Actinoplanes*, which could effectively degrade organic matter and fix nitrogen. We also showed that the organic matter, total nitrogen and C / N ratio were positively correlated with the relative abundance of functional microbes in rice-rape rotation farmland in Erhai basin. These results provide empirical evidence for the utility of different organic fertilizers in improving soil fertility and crop yield.

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