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Changes in soil microbial community composition in response to fertilization of paddy soils in subtropical China

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ABSTRACT

Repeated fertilizer applications to cultivated soils may alter the composition and activities of microbial communities in terrestrial agro-ecosystems. In this study, we investigated the effects of different long term fertilization practices (control (CK), three levels of mineral fertilizer ($N_1P_1K_1$, $N_2P_2K_2$, and $N_3P_3K_3$), and organic manure (OM)) on soil environmental variables and microbial communities by using phospholipid fatty acid (PLFA) biomarkers analysis in subtropical China. Study showed that OM treatment led to increases in soil organic carbon (SOC), total nitrogen (TN) and total phosphorus (TP) contents, while the mineral fertilizer treatment led to increases in dissolved organic carbon (DOC) content. Changes in soil microbial communities (eg. bacteria, actinomycetes) were more noticeable in soils subjected to organic manure applications than in the control soils or those treated with mineral fertilizer applications. Fungal PLFA biomarkers responded differently from the other PLFA groups, the numerical values of fungal PLFA biomarkers were similar for all the OM and mineral fertilizer treatments. PCA analysis showed that the relative abundance of most PLFA biomarkers increased in response to OM treatment, and that increased application rates of the mineral fertilizer changed the composition of one small fungal PLFA biomarker group (namely 18:3ω6*c* and 16:1ω5*c*). Further, from the range of soil environmental factors that we examined, SOC, TN and TP were the key determinants affecting soil microbial community. Our results suggest that organic manure should be recommended to improve soil microbial activity in subtropical agricultural ecosystems, while increasing mineral fertilizer applications alone will not increase microbial growth in paddy soils.

1. Introduction

Soil microorganisms play an important role in the soil environment. They are the critical factors that determine soil organic matter decomposition, nutrient cycling, soil degradation and bioremediation of soil pollution (Larkin, 2003; Li et al., 2012). Shifts in the structure and composition of the microbial community are strong indicators of soil biological activity, soil quality and crop productivity of terrestrial agro-ecosystems (Edmeades, 2003).

China is one of the major rice cultivation countries in the world, producing approximately one-third of the global rice crop (Coats, 2003). The cultivation of rice, interchanging between dry and wet field conditions, gives rise to anoxic conditions when soils are flooded during most of the rice-growing season and aerobic conditions when soils are drained during the non-cropping season (Li et al., 2010). Thus, the composition and structure of microorganisms in paddy soils are diverse and complicated (Ge et al., 2008).

Research has clearly demonstrated that environmental conditions and soil management practices largely determine the structure of soil microbial communities (Steenwerth et al., 2002; Wu et al., 2011). Paddy soils are routinely fertilized to improve soil nutrition and maximize the rice yield. Just as different groups of microorganisms vary in their ability to adapt to the various soil nutrient conditions, fertilization will also certainly influence soil microbial growth and activity (Broeckling et al., 2008; Wei et al., 2008). Repeated fertilizer applications to soil can change the soil microbial community directly or indirectly since they change the soil physical, chemical and biological properties (Beauregard et al., 2010).

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Some studies have documented that fertilization has had significant impacts on the population, composition and function of soil microorganisms, and that organic and inorganic fertilizer amendments have increased the soil microorganisms' activity (Ge et al., 2008; Mandal et al., 2007). However, other studies have demonstrated that inorganic and organic fertilizers have had relatively little or no effect on soil microbial diversity and activities (Okano et al., 2004; Treseder, 2008). Long-term fertilization experiments can be controlled so as to modify soils in a particular manner and so can contribute significantly to existing knowledge about the evolution of soil fertility, the effects of fertilization, nutrient cycling in croplands, as well as soil biogeochemical cycles. Thus, research on soil microorganism communities under long-term fertilizer management has been one of the foci of soil ecological research in recent years (He et al., 2008; Yamaguchi et al., 2009).

Phospholipid fatty acids (PLFAs) are a vital component of the membrane (essentially the skin) of all microbes, and their polar head groups and ester-linked side chains (i.e. FAs) vary in composition between eukaryotes and prokaryotes, as well as among many prokaryotic groups (Drenovsky et al., 2004; Hao et al., 2008). These compounds rapidly degrade as cells die, making them good indicators of living organisms (White et al., 1979). Thus, analysis of microbial populations using PLFA analysis provides direct information for the identification, classification and quantification of microbial community composition which overcomes the selectivity problems associated with culture techniques (Wu et al., 2009a).

In the paddy soils of subtropical China, long-term fertilizer trials were conducted to study the effects of fertilization on soil microbial communities (Beauregard et al., 2010; Zhang et al., 2012). However, most of these long-term studies have focused on different mineral fertilizer application rates, and the effects of long-term applications of different types of fertilizers on the soil microbial community structures remain poorly understood. In this study, we collected soil samples from a 13-year fertilization experiment, during which time mineral fertilizer and organic manure amendments were applied to an agricultural soil in subtropical China. We hypothesized that (i) the effects of fertilization on soil microbial community composition would vary with the fertilizer type; (ii) the organic manure would increase the abundance of the main soil microbial communities (e.g. bactertia and fungi); while different mineral fertilizer rates may have positive or negative effects on soil microbial communities (e.g. bactertia and fungi) by altering soil properties. We investigated the effects of different long term fertilization practices (CK, organic manure, different rate of mineral fertilizer) on soil microbial communities using phospholipid fatty acid (PLFA) analysis. The aims of this study were to (i) evaluate the effects of organic and mineral fertilizer treatments on the community structure of soil microorganisms and (ii) investigate the influence of soil chemical properties on soil microbial communities.

2. Materials and methods

2.1. Study site

The experiment was conducted at the Chinese Academy of Sciences Qianyanzhou Experimental Station (115°03′29.2″E, 26°44′29.1″N), located in the hill red-earth region of South China. The region has a subtropical monsoon climate. According to the meteorological statistics, the mean annual temperature is 17.8 °C, and the annual active accumulated temperatures (above 0 and 10 °C) are 6543.8 and 5948.2 °C, respectively. The annual precipitation and evaporation are 1471.2 mm and 259.9 mm, respectively, and the mean relative humidity is 83%. The frost-free period is 290 days and global radiation is 4223 MJ m^{-2}. Our experimental site was located in the flat floodplain where the soil-forming parent material consists of red sandstone and sandy conglomerate. Investigation and analysis before our experiment showed that the main soil type was paddy soils, the bulk density of which was 1.50 g cm^{-3} (0–20 cm), pH was 5.97, SOC content was 9.71 g kg^{-1}, the total N content was 1.02 g kg^{-1}, and available P was 1.6 mg kg^{-1}.

2.2. Experimental design

A long-term fertilizer experiment was initiated in 1998 under a double rice cropping system (rice–rice–winter fallow), which is one of the most common cropping systems in the region. In this system, summer rice is sown at the end of April and harvested in July, and winter rice is sown at the end of July and harvested in November. During the growing season, we used the same field practices (field preparation, tillage, puddling, and irrigation) as the local farmer. No pesticides were applied during the growing season and weeds were controlled manually.

We analyzed samples from five different treatments: (1) CK (unfertilized control), (2) $N_1P_1K_1$ (mineral N, P, K fertilizers applied as N–P_2O_5–K_2O at a rate of 225–135–225 kg ha^{-1}, (3) $N_2P_2K_2$ (mineral N, P, K fertilizers applied as N–P_2O_5–K_2O at a rate of 450–270–450 kg ha^{-1}, (4) $N_3P_3K_3$, mineral N, P, K fertilizers applied as N–P_2O_5–K_2O at a rate of 675–405–675 kg ha^{-1}, (5) OM (organic manure) applied at a rate of 41,000 kg ha^{-1} fresh weight (N content is 0.55%). In the NPK treatments, N, P, and K were applied as urea, calcium–magnesium phosphate, and potassium chloride. The organic manure used in the OM treatment was comprised of pig feces, which was collected from a nearby pig farm in Taihe County. Prior to being applied as a manure, it was subjected to a high temperature composting-process, which resulted in a good organic fertilizer after a few months of fermentation, sterilization, and deodorization (Zhang et al., 2009). All fertilizer was applied twice a year: 44% was applied in April for early rice and 56% was applied in July for late rice.

Treatments were arranged in a randomized block design with three replications (Bi et al., 2009; Sikka and Kansal, 1995), totaling 15 plots. Each plot was 15 m^2 (3 m × 5 m) and was isolated by concrete walls (50 cm depth and 15 cm above the soil surface). These fertilization systems were chosen as they were consistent with methods used by local farmers.

2.3. Sampling collection and analysis

Soil samples were collected from the 15 plots at 10 days after the late rice harvest in 2011. In each plot, soils were sampled in the plow layer (0–20 cm) using an auger with a 5 cm internal diameter at five randomly selected locations and then mixed as one sample. All the fresh soil samples were air-dried and sieved twice using 2.0 mm and 0.25 mm meshes and stored for nutrient analysis. Soil chemical properties were measured using the methods described by Bao (2005). Soil pH was measured with a glass electrode in a 1:2.5 soil/water suspension. The dissolved DOC content of filtered 0.5 M K_2SO_4 extracts from fresh soil was measured with a TOC analyser (Liqui TOC Elementar, Vario Max, Germany). Concentrations of NH_4^+-N and NO_3^--N of filtered 2 M KCl extracts from fresh soil were measured with a flow injection autoanalyser (AutoAnalyzer 3, Bran + Luebbe, Germany). Concentrations of C and N were measured with an elemental analyzer (Elementar, Vario Max, Germany) (Huang et al., 2012). Total soil P was analyzed with a flow injection autoanalyser following H_2SO_4–$HClO_4$ digestion (Benke et al., 2010).

The soil microbial community was characterized using phospholipid fatty acid (PLFA) analysis. Phospholipid fatty acid (PLFA) was extracted from the soil using the procedure of Bossio and Scow

(Bossio and Scow, 1998). Total lipids were extracted from 8 g of soil sample using buffers of potassium phosphate, chloroform, and methanol. Phospholipids were fractionated from neutral and glycolipids on a silica column. After mild alkaline methanolysis to form fatty acid methyl esters (FAMEs), samples were then dissolved in hexane and analyzed in an Agilent 6890N gas chromatograph with MIDI peak identification software (version 4.5; MIDI Inc. Newark, DE) and an Agilent 19091B-102 (25.0 m × 200 μm × 0.33 μm) capillary column. The carrier gas was hydrogen. The GC temperature progression was set by the MIDI software. The fatty acid 19:0 was added as an internal standard before methylation and fatty acid methyl esters were identified automatically by the MIDI peak identification software (Wu et al., 2009b).

We used the following fatty acid nomenclature: total number of carbon atoms: number of double bonds, followed by the position (ω) of the double bond from the methyl end of the molecule. Cis and trans configurations are indicated by *c* and *t*, respectively. Anteiso- and iso-branching are designated by the prefix a or i. 10Me is a methyl group on the 10th carbon atom from the carboxyl end of the molecule (Bååth and Anderson, 2003).

Total amounts of the different PLFA biomarkers were used to represent the different groups of soil micro-organisms. The sum of the following PLFA biomarkers were considered to represent bacterial origin (gram-positive bacteria by i14:0, i15:0, a15:0, i16:0, a16:0, i17:0, a17:0, gram-negative bacteria by 16:1ω9*c*, cy17:0, 18:1ω5*c*, 18:1ω7*c*, cy19:0, bacteria were represented by the sum of the two) (Frostegård and Bååth, 1996). Biomarkers 18:3ω6*c*, 18:1ω9*c* and 16:1ω5*c* were used as a marker for fungal PLFA, and 10Me16:0, 10Me17:0 and 10Me18:0 were used for indicating actinomycetes PLFA (Zelles, 1997). Taken together, all of the PLFA biomarkers indicated above were considered to be representative of the total PLFA of the soil microbial community.

2.4. Statistical analysis

All the results were reported as means ± standard error (SE) for three replicates. We used one-way analysis of variance (ANOVA) to identify differences in the responses of soil physio-chemical characteristics and soil microbial biomass to fertilization, and applied a significance level of $P < 0.05$. PLFA biomarkers obtained from the sampled soils were compared and analyzed by principal component analysis (PCA). Redundancy analysis (RDA) was applied to investigate the responses of soil microbial parameters to environmental variables using CANOCO software version 4.5 for Windows. Automatic selection of means by Monte Carlo permutations was used to test the significance of the variables.

3. Results

3.1. Effects of the different treatments on soil chemical properties

Soil chemical properties changed substantially after 13 years of different fertilizer applications (Table 1). pH, DOC, TN, SOC and TP contents were significantly higher in soils treated with fertilizers when compared with the CK treatment ($P < 0.05$). In particular, the pH was highest in the $N_2P_2K_2$ treatment (6.9), and was 28% higher than for the CK. The $N_3P_3K_3$ treatment resulted in the highest DOC content (216.0 mg kg^{-1}), and was 33% higher than for the CK. In addition, SOC, TN and TP contents in soils treated with OM were notably higher than in soils from the other treatments ($P < 0.05$), and were approximately 2.5, 2.0 and 4.4 times that of the CK, respectively. However, there were no apparent differences between the NO_3^--N (ranging from 1.2 to 5.4 mg kg^{-1}) and NH_4^+-N (ranging from 9.1 to 13.6 mg kg^{-1}) concentrations among the five treatments.

3.2. Effects of the different treatments on the soil microbial community composition

3.2.1. Total PLFAs

Microbial biomass, estimated as total PLFA biomarkers, showed significant differences ($P < 0.05$) among the different treatments following 13 years of fertilizer applications (Fig. 1). We observed that the applications of OM and NPK led to a marked increase in the total PLFA biomarker concentrations in the soil. Total PLFA biomarkers increased by 85% under the OM treatment when compared with the CK, while there was a 49% increase in total PLFA biomarkers for the $N_2P_2K_2$ treatment. However, the total PLFA biomarkers were not significantly different between the different NPK treatments.

3.2.2. Bacterial community

The addition of organic manure and NPK fertilizers led to a significant increase in the bacterial biomass of all soils compared to CK ($P < 0.05$). Specifically, the bacterial PLFA biomarkers in the OM

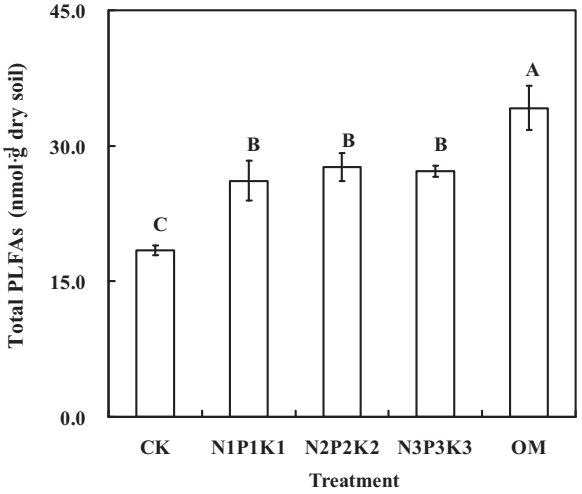

Fig. 1. Abundance of soil total PLFAs under different fertilization treatments. Bars represent means ± standard error. The different letter on the top of the column indicates a statistically significant difference between the treatments at $P < 0.05$ level using one-way ANOVA.

Table 1
Chemical properties of soils sampled under different fertilizer treatments (means ± standard error).

Treatments	pH	DOC (mg kg^{-1})	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	SOC (g kg^{-1})	TN (g kg^{-1})	TP (mg kg^{-1})
CK	5.4 ± 0.1c	162.5 ± 12.6b	1.2 ± 0.2ns	9.1 ± 0.6ns	8.5 ± 0.5c	0.9 ± 0.0b	117.6 ± 1.6b
$N_1P_1K_1$	6.1 ± 0.1b	211.7 ± 3.6a	2.3 ± 0.5ns	11.3 ± 0.2ns	17.5 ± 1.3a	1.6 ± 0.1a	349.1 ± 58.4ab
$N_2P_2K_2$	6.7 ± 0.0a	215.9 ± 6.4a	3.5 ± 0.9ns	12.5 ± 2.1ns	14.9 ± 0.6b	1.7 ± 0.1a	461.0 ± 32.8a
$N_3P_3K_3$	6.9 ± 0.0a	178.9 ± 8.8ab	5.4 ± 1.1ns	13.6 ± 0.3ns	14.4 ± 0.3b	1.6 ± 0.1a	501.3 ± 85.2a
OM	6.0 ± 0.2b	204.9 ± 15.0a	3.3 ± 1.3ns	11.1 ± 0.6ns	21.1 ± 2.1a	1.8 ± 0.1a	518.4 ± 108.0a

DOC, dissolved organic carbon; SOC, total soil organic carbon; TN, total soil nitrogen; TP, total soil phosphorus. Different letters in the same column indicate a significant difference at $P < 0.05$; ns, no significant difference between treatments using ANOVA.

treatment (21.14 nmol g^{-1}) were 89% higher than in the CK treatment (11.18 nmol g^{-1}) (Fig. 2a). Though greater than CK treatment values, there were no significant differences among the three levels of NPK treatments. The abundance of gram-positive (G^+) bacterial biomarkers were only slightly higher than that of gram-negative (G^-) bacterial biomarkers in all the treatments, but changes in the abundance of G^+ and G^- bacterial biomarkers followed the same pattern as total bacterial PLFA biomarkers with regards to treatment (Fig. 2c–d). The highest G^+ bacterial and G^- bacterial contents were observed in the OM treatment (11.75 and 9.39 nmol g^{-1}), but there were no significant differences among the three levels of NPK treatments (ranging from 8.55 to 9.10 nmol g^{-1} and 7.11 to 7.75 nmol g^{-1}, respectively). The ratios of G^+/G^- PLFA biomarkers were consistent across treatments at approximately 1.2 (Fig. 2b).

3.2.3. Fungal community

Applications of organic manure and NPK fertilizer improved the fungal activity in soils (Fig. 3). The fungal PLFA biomarkers in $N_1P_1K_1$, $N_2P_2K_2$, $N_3P_3K_3$ and OM treatments were 41%, 38%, 33% and 59% higher than in the CK ($P < 0.05$), respectively, whereas there were no significant differences between the fungal PLFA biomarker contents in all the NPK mineral fertilizer treatments and OM treatments. Further, the effects of the fertilizer treatments on fungal to bacterial (F/B) ratios were not significant ($P > 0.05$), the values of which were close to 0.20.

3.2.4. Actinomycete community

The changes in the actinomycete PLFA biomarker contents for the five treatments followed similar patterns to the changes in total and bacterial PLFA biomarkers in our study (Fig. 4). There were significant differences in the actinomycete PLFA biomarker contents between different fertilizer treatments ($P < 0.05$). The highest actinomycete PLFA biomarker content was found in soils treated with OM (6.77 nmol g^{-1}), which was about 1.5 times that found for the CK (3.30 nmol g^{-1}).

3.3. Principal component analysis

Effects of the different fertilizer amendments on PLFA biomarker composition were evaluated by principal component analysis (PCA). The first principal component (PC1) accounted for 63.5%, and the second component (PC2) for 16.4% of the variation in the profiles (Fig. 5). During the experimental period, the significant effects of the fertilizer on PLFA showed an obvious sequential shifting along the PC1 axis in the order OM – $N_3P_3K_3$ – $N_2P_2K_2$ – $N_1P_1K_1$ – CK. The OM treatments with higher PC1 scores were observed on the right of the PC1 axis, whereas the CK and $N_1P_1K_1$ treatments with lower scores were observed mostly on the left of the PC1 axis. The samples from the $N_2P_2K_2$ and $N_3P_3K_3$ treatments grouped together as a separate cluster (Fig. 5a).

Fig. 5b shows the loading values for the individual PLFA biomarkers under the different fertilizer treatments. It should be

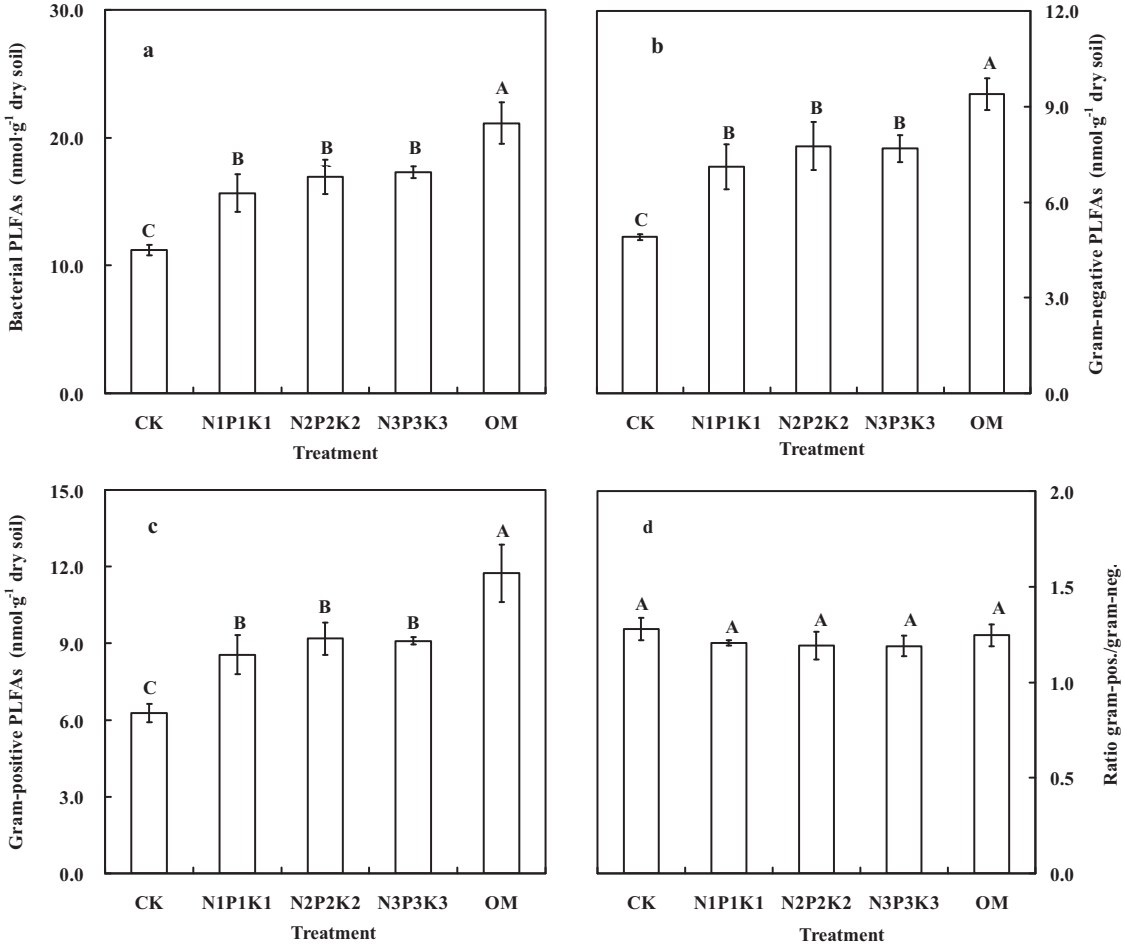

Fig. 2. Abundance of soil bacterial, gram-positive, gram-negative PLFAs and ratio of gram-positve to gram-negative PLFAs under different fertilization treatments. Bars represent means ± standard error. The different letter on the top of the column indicates a statistically significant difference between the treatments at $P < 0.05$ level using one-way ANOVA.

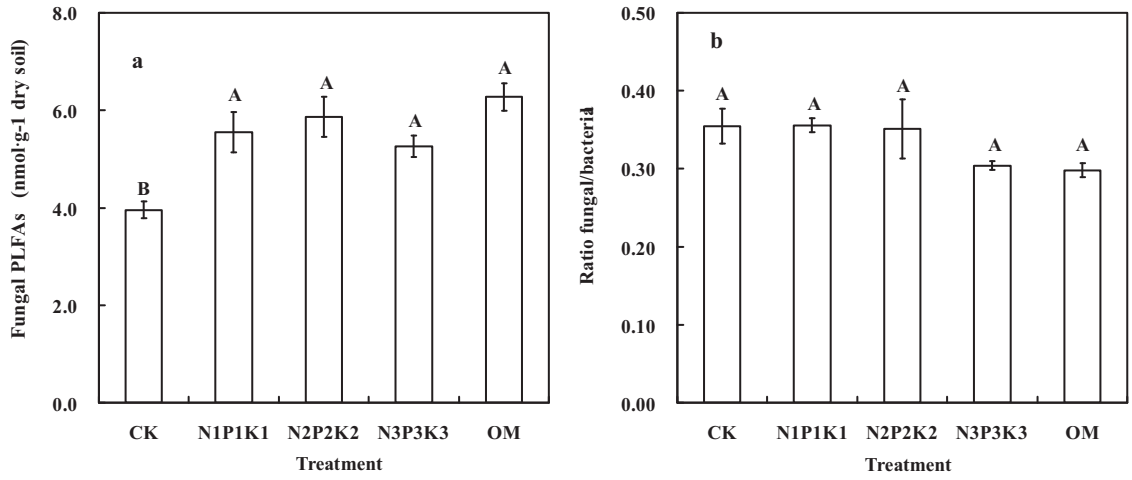

Fig. 3. Abundance of soil fungal PLFAs and ratio of fungal to bacterial PLFAs under different fertilization treatments. Bars represent means ± standard error. The different letter on the top of the column indicates a statistically significant difference between the treatments at $P = 0.05$ level using one-way ANOVA.

noted that the PLFA biomarkers associated with bacteria, including i14:0, a15:0, i15:0, i16:0, i17:0, a17:0, cy17:0, cy19:0 and 18:1ω7*c*, were all more abundant under the OM treatment. In particular, applications of NPK fertilizers led to an increase in two of the PLFA biomarkers (namely 18:3ω6*c* and 16:1ω5*c*), indicating eukaryotic organisms, as well as one of the bacterial PLFA biomarkers (represented by 16:1ω9*c*). The loading values of actinomycete PLFA biomarkers indicate that the fatty acid 10Me16:0, 10Me17:0 and 10Me18:0 were most important for the OM treatment, as the relative abundance of these three fatty acids was the highest in this treatment.

3.4. Redundancy analysis

The RDA ordination biplot (Fig. 6) shows the relationship between the environmental variables and the soil PLFA contents. The first ordination RDA axis (horizontal) was mainly correlated with SOC, TN and TP contents and explained 88% of the total variability of the PLFA. The second ordination axis (vertical), which was strongly associated with pH, explained 1.5% of the total variability. According to the results of the Monte Carlo permutation test, the contents of TN, SOC and TP ($P < 0.05$) were the major factors explaining variations of PLFA levels under the experimental conditions assayed in the study. The ratio of F/B correlated positively with pH and NO_3-N and correlated negatively with other environmental variables. However, the contents of bacterial PLFA biomarkers, fungal PLFA biomarkers, actinomycete PLFA biomarkers and total PLFA biomarkers correlated positively with all the environmental variables.

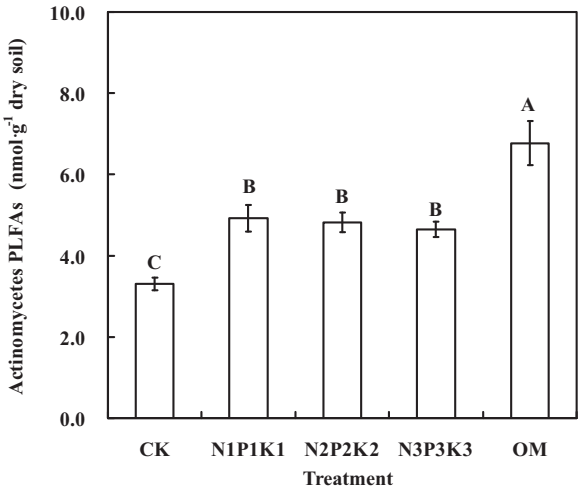

Fig. 4. Abundance of soil actinomycetes PLFAs and ratio of fungal to bacterial PLFAs under different fertilization treatments. Bars represent means ± standard error. The different letter on the top of the column indicates a statistically significant difference between the treatments at $P = 0.05$ level using one-way ANOVA.

4. Discussion

4.1. Effects of treatments on the soil chemical properties

A large number of long-term experiments have been initiated worldwide to evaluate the effects of long-term fertilizer applications on the fertility of paddy soils in rice cropping systems (Ladha et al., 2003; Whitbread et al., 2003). Many of these studies were also set up to monitor changes in soil fertility in response to fertilization in subtropical China (Cong et al., 2012). However, results from previous studies have been variable. Some studies have documented that long-term fertilizer applications lead to enhanced soil fertility (Shen et al., 2004; Whitbread et al., 2003), while other studies have reported that long-term fertilization has caused severe degradation of red soils, characterized by high acidity, low nutrients and a disturbed, unbalanced ecosystem (Dawe et al., 2003; Kumar and Yadav, 2001).

In this present study, the application of NPK fertilizers and organic manure resulted in increase in soil pH compared to control conditions, which suggests that mineral fertilizer and organic manure could, to some extent, alleviate soil acidification. This may be facilitated by the application of alkaline phosphate fertilizer (e.g. calcium magnesium phosphate) which would increase the soil pH (Wu et al., 2009b). In addition, the application of organic manure may improve soil acidity by increasing the soil organic matter, promoting soil maturation, and enhancing the soil base saturation percentage, which is in agreement with the results of Li et al. (Li et al., 2010).

We found that the application of organic manure and NPK fertilizers resulted in obvious changes in chemical properties. It is already well known that long term applications of organic manure are beneficial for the accumulation of soil organic matter and thus improve different aspects of soil fertility (Hyvönen et al., 2008; Liang et al., 2012). In line with this concept, our results showed that organic manure resulted in increased SOC, TN and TP contents in the soil. However, concentrations of NH_4^+-N and NO_3^--N did not differ significantly under different fertilizer treatments. This may

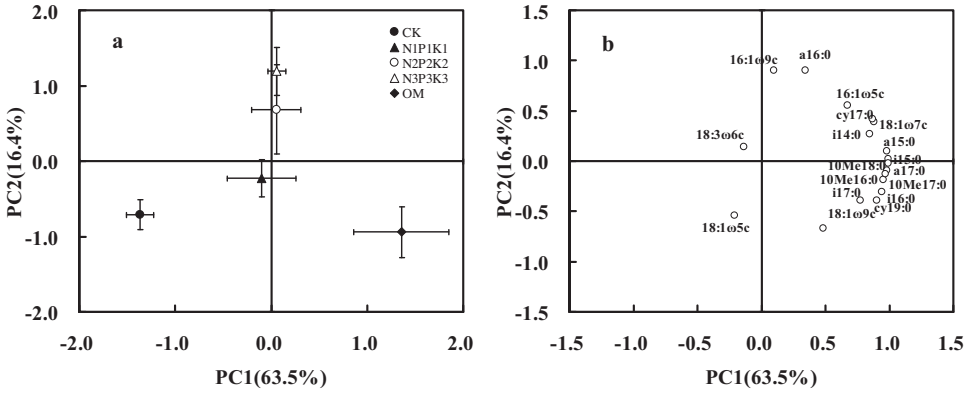

Fig. 5. Principal component analyses (PCA) of the PLFA profiles in the soil samples from different fertilization treatments. (a) Score plot of the different fertilization treatments. (b) Loading values of the individual PLFA. Bars represent means ± standard error.

be because the soil would be continually changing from states of oxidation and reduction under the wetting and drying cycles experienced in the rice field conditions, and the soil moisture would influence the NH_4^+-N and NO_3^--N content of the soil (Xiong et al., 2010). Also, pools of NH_4^+-N and NO_3^--N are much smaller relative to SOC, TN, and TP. Thus, transient (i.e. seasonal) changes in these small, highly active, pools may mask larger systemic changes in carbon and nutrient dynamics (that are reflected in SOC, TN, and TP) (Galvez et al., 2011).

4.2. Effects of treatments on the soil microbial community composition

In our study, application of organic manure resulted in larger populations of total, bacterial and actinomycete, but not fungal PLFA biomarkers. This result suggested that the effects of organic manure application had a stronger influence on soil microbial communities (e.g. bacteria, actinomycete) than either no treatment or mineral fertilizer applications. Long term applications of organic manure may play an important role in sustaining soil microbial activities (Li et al., 2007). Organic amendments may provide a greater diversity of potential substrates for microbe growth and reproduction (Zeng et al., 2007). Further, the microorganisms occurring naturally in the organic manures could also be contributing to the enhanced microbial biomass in soil. A study by Chu et al. (2007) demonstrated that organic manure had a significantly greater impact on the biomass C and the microbial activity, and that balanced fertilization also resulted in higher microbial metabolic activity than nutrient-deficiency fertilization in soil. However, other studies show that organic manure does not seem to be important for determining microbial biomass, as it can vary depending on the organic manure type and application time (De Vries et al., 2006).

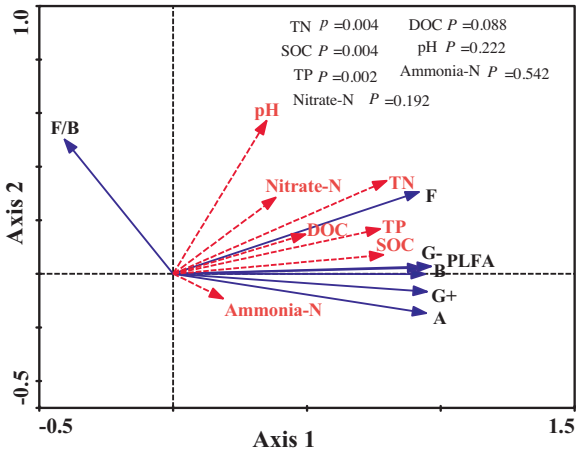

Fig. 6. RDA-ordination biplot of PLFA profiles in the soil samples and environmental variables. The explanatory variables are indicated by different arrows: PLFA profiles by solid arrows: bacterial PLFAs (B); gram-positive bacterial PLFAs (G+); gram-negative bacterial PLFAs (G−); fungal PLFAs (F); actinomycete PLFAs (A); total PLFAs (PLFA), environmental variables by dashed arrow: dissolved organic carbon (DOC); total soil organic (SOC) carbon; total soil nitrogen (TN); total soil phosphorus (TP).

In this current study, mineral fertilizer practices had a positive impact on the communities of specific microbial groups. But the form of fertilizer (inorganic vs. organic) was more important than the amount of NPK added. In the broader scale, the effects of mineral fertilizer on the soil microbial community composition are just as uncertain as those of organic fertilizer. Numerous studies have demonstrated that shifts in soil microbial community composition may occur as a result of fertilizer amendment (Clegg et al., 2006; Kennedy et al., 2004), however the effects of these interactions between mineral fertilizer transformations on the composition of soil microbial communities are not clear. Böhme et al. (2005) reported that the total PLFA biomass abundance was quite different in soils where mineral fertilizers were added from site to site: some was higher than unfertilized land, and some were lower. This may be related to differences in soil environmental conditions (e.g. temperature, humidity) and plant species composition. Lovell et al. (1995) suggested that microbial biomass was related to the long-term C input: long-term applications of high rates of fertilizer led to a reduction in the C input from sward root mass, and a consequently a lower microbial biomass.

Fungi play a significant role in carbon and nutrient cycling in terrestrial ecosystems and are known to be sensitive to biofertilizers (Deacon et al., 2006). Our results showed that different fertilizer treatments had no obvious effects on soil fungal communities and that the fungal PLFA appeared to be approximately the same in all the fertilizer treatments. De Vries et al. (2006) also found that soil fungi were not significantly affected by different fertilization treatments, and may reflect the fact that invasive land management practices (such as fertilization) decreased fungal activity while similar soils that were less intrusively managed had higher fungal activity. In addition, applications of mineral fertilizer promoted growth of microbial communities that were limited by mineral nutrients, which also caused a shift in the factors that were limiting for microbial growth such as organic C, and so were not conducive to maintaining soil microbial populations (Parham et al., 2003).

The F/B ratio, estimated by the fungi and bacterial biomass in soil, was used as a measure of their responses to environmental change and the functioning of the soil microbial communities (Strickland and Rousk, 2010). It has been suggested that a higher F/B ratio is indicative of a more sustainable agro-ecosystem with lower environmental impact, in which organic matter decomposition and N mineralization dominate the provision of plant nutrients for crop growth (De Vries et al., 2006). Previous studies have pointed out that one of the most important characteristics likely to influence the F/B ratio is nutrient availability (Suzuki et al., 2009). It has been reported that mineral fertilizers reduce the F/B ratio, while organic manure with high C/N ratio stimulate fungi growth and thus increase the F/B ratio (Buyer et al., 2010). Our study however showed that fertilizer could promote soil C storage due to an increase in total microbial biomass. Bardgett and McAlister (1999), who found that fertilizer did not lead to a significant increase in fungal/bacterial dominance after 6 years, hypothesized that this may have been due to high residual fertility. Similar results were obtained in our study: there were no significant differences between the F/B ratios for any of the treatments, but rather they all showed similar trends.

4.3. Effects of treatments on the soil PLFA distribution

Examination of the relative contribution of the individual PLFA biomarkers showed that different fertilizer practices have an impact on the community structure of specific microbial groups, and that organic manure and mineral fertilizers may shift microbial functional groups in the opposite direction. Most of the variation in PLFA biomarker patterns was due to different fertilizer amendments and could be explained by the first two principal components. Data from our experiment also revealed that organic manures led to an increase in a group of PLFA biomarkers associated with bacteria, primarily the anteiso- and iso-fatty acids (i14:0, a15:0, i15:0, i16:0, i17:0, a17:0) and a number of gram-positive bacterial biomarkers (cy17:0, cy19:0 and 18:1ω7c). This is consistent with the previous studies which showed that gram-negative microbial biomarkers (viz., monoun-saturated fatty acid) were more reactive to the organic manure (Bossio and Scow, 1998; Zhang et al., 2012). Furthermore, examination of individual fungal PLFA biomarkers indicated that part of the fungal growth was facilitated by mineral fertilizer and inhibited by organic manure, suggesting the high prevalence of fungi in the paddy soils may be have been related to the high mineral fertilizer application rates and the low organic manure application rates. Previous research has shown that 10Me16:0, 10Me17:0 and 10Me18:0 fatty acids (common in actinomycete) were more abundant in unfertilized soil than in the organic matter amended soils (Clegg et al., 2006; Lundquist et al., 1999). In contrast, the PC loading analysis in our study showed that actinomycete viz., 10Me fatty acid grew more rapidly in organic manure amended soil than in mineral fertilizer amended soil.

Data from this study show that the soil microbial community both had an influence on and was influenced by soil chemical properties. The RDA analysis illustrated the relationships and differences in microbial community structure and soil chemical properties among the treatments (Fig. 6) and showed that environmental variables measured in this study explained 89.8% of the total variability of the soil microbial community composition in paddy soils. The soil microbial community can be considered as a sensitive element that regulates the chemical environment of the earth through metabolic activities such as respiration, mineralization and denitrification (Cruz et al., 2009). Moreover, the components of the soil microbial community (e.g. bacteria, fungi and actinomycete) tended to be positively correlated with all the environmental variables, which suggest that nutrient recycling and soil microbial activity in rice field ecosystems are highly dependent upon each other. Various studies have shown that microbial growth and activity are generally limited by C, N and P availability (Vineela et al., 2008). Our results provided further evidence that SOC, TN, and TP were key factors for microbial growth in subtropical paddy soils, and were strongly related to microbial biomass and activity. Hence, the influence of the nutrient balance in determining the soil microbial community deserves further study in order to understand how to make better use of fertilizer and enhance its efficiency in subtropical paddy soil regions.

Soil pH is a major factor influencing the structure of the soil microbial community (Nilsson et al., 2007). For example, a study of the effects of latitude on microbial community structure showed that the increase of pH caused a shift in the soil microbial community (Wu et al., 2009b). In the present study, soil total PLFA biomarkers were also positively correlated with soil pH. Similarly, the F/B ratio of increased as the soil pH increased, which according to other studies, indicates more sustainable agro-ecosystem function (Bardgett and McAlister, 1999). Nonetheless, the F/B ratio was negatively correlated with soil nutrients, which might be because the bacteria in high fertility paddy soils were more markedly enriched than fungi.

In conclusion, the results of this study demonstrate that long-term fertilization with mineral fertilizer or organic manure promoted the growth of bacteria, actinomycete and total microbial organisms, whereas taken individually, the addition of mineral fertilizer was less effective than manure. PCA analysis showed that the relative abundance of most PLFA biomarkers increased in response to the OM treatment, and that an increase in mineral fertilizer application rates would change the composition of a few groups of the PLFA biomarkers, which indicates that the applications of mineral fertilizer alone are not sufficient to sustain microbial growth in paddy soils. Organic manure should be recommended to improve soil microbial activity and thereby sustain the microbial environment of paddy soils. Further, SOC, TN and TP contents had obvious effects on the PLFA biomarkers in all the treated soils, suggesting that they were the key variables for maintaining the soil microbial community.

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