Impacts of long-term fertilization on the soil microbial communities in double-cropped paddy fields

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Abstract

The response of soil microbial communities to soil quality changes is a sensitive indicator of soil ecosystem health. The current work investigated soil microbial communities under different fertilization treatments in a 31-year experiment using the phospholipid fatty acid (PLFA) profile method. The experiment consisted of five fertilization treatments: without fertilizer input (CK), chemical fertilizer alone (MF), rice (*Oryza sativa* L.) straw residue and chemical fertilizer (RF), low manure rate and chemical fertilizer (LOM), and high manure rate and chemical fertilizer (HOM). Soil samples were collected from the plough layer and results indicated that the content of PLFAs were increased in all fertilization treatments compared with the control. The iC15:0 fatty acids increased significantly in MF treatment but decreased in RF, LOM and HOM, while aC15:0 fatty acids increased in these three treatments. Principal component (PC) analysis was conducted to determine factors defining soil microbial community structure using the 21 PLFAs detected in all treatments: the first and second PCs explained 89.8% of the total variance. All unsaturated and cyclopropyl PLFAs except C12:0 and C15:0 were highly weighted on the first PC. The first and second PC also explained 87.1% of the total variance among all fertilization treatments. There was no difference in the first and second PC between RF and HOM treatments. The results indicated that long-term combined application of straw residue or organic manure with chemical fertilizer practices improved soil microbial community structure more than the mineral fertilizer treatment in double-cropped paddy fields in Southern China.

Introduction

Soil microorganisms are the most sensitive and rapid indicators of perturbations and land use changes. The composition of microbial communities, in particular the proportion of bacteria and fungi, may influence carbon (C) and nitrogen (N) turnover of paddy soils. The quantitative description of microbial community structure and diversity is therefore of great interest as a potential tool for soil quality evaluation (García-Orenes *et al.*, 2013). The cell membranes of organisms, including soil organisms such as fungi and bacteria, are composed mainly of phospholipids. Cell membranes can be extracted and decomposed into their constituent fatty acids (phospholipid-derived fatty acids or PLFAs) for analysis. These are good indicators of living organisms and can be used to detect rapid changes in soil microbial communities because they are essential components of the membranes of all living microbes, and they degrade rapidly as cells die (White *et al.*, 1979). Furthermore, different PLFAs can be used as biomarkers for major microbial groups, e.g. fungi and bacteria (Hao *et al.*, 2008).

In recent years, research has reported that many environmental factors influence soil microbial communities, including soil texture (Chodak and Niklińska, 2010), nutrient availability (Bowles *et al.*, 2014), land use (Kong *et al.*, 2006), water content (Moche *et al.*, 2015) and temperature (Wang *et al.*, 2014). It is generally recognized that soil microbial communities and diversity are related closely to different fertilization practices (Williams *et al.*, 2013; Zhang *et al.*, 2016; Hu *et al.*, 2017). It has been reported that long-term application of chemical N fertilizer to a fluvo-aquic soil in Northern China increased fungal abundance but decreased bacterial abundance (Ai *et al.*, 2012). Some studies have demonstrated that mineral fertilizers increased soil microbial biomass and diversity (Zhong *et al.*, 2010). However, some other studies found that fungal and bacteria communities were decreased by long-term application of N and P fertilization (Demoling *et al.*, 2008; Zhou *et al.*, 2016). Williams *et al.* (2013) pointed out that soil microbial community composition was decreased under long-term mineral fertilization. Yevdokimov *et al.* (2012) reported that the ratio between PLFAs of Gram-positive bacteria (G$^+$ bacteria) and Gram-negative bacteria (G$^-$ bacteria) decreased under the application of N fertilizer at a rate of 2000 mg N/kg soil. Organic fertilizers,

including plant residues, animal manure and composted organic matter, are usually found to increase soil microbial activity and diversity (Islam *et al.*, 2011), but the extent of the increase depends on the type and quantity of amendments. Zhang *et al.* (2016) found that soil microbial communities were influenced directly by residue return and further reported that the ratio of fungi to bacteria decreased when the amount of residue application increased. It has been reported that organic manure application could significantly alter soil microbial community structure and enhance microbial diversity (Dong *et al.*, 2014; Li *et al.*, 2015; Sun *et al.*, 2016; Hu *et al.*, 2017). Tian *et al.* (2017) also found that abundance of soil PLFAs, bacteria, G$^+$ bacteria and actinomycetes was higher with manure than the combined application of manure with mineral fertilizer. Ai *et al.* (2012) reported that ratios of total PLFAs and G$^+$ bacteria to G$^-$ bacteria in fluvo-aquic soil were increased by application of organic fertilizer.

Many studies have demonstrated that soil microbial community structure was changed under different agricultural management practices, such as crop rotation (Kong *et al.*, 2006), land management (Yu *et al.*, 2013), soil type (Demoling *et al.*, 2008), soil tillage (Bossio *et al.*, 1998) and residue return (Zhang *et al.*, 2016), and there have been several studies on the effects of different fertilizer regimes on soil microbial community structure in paddy fields (Islam *et al.*, 2011; Dong *et al.*, 2014). However, information about the effects of long-term fertilization practices on soil microbial community structure is limited in double-cropped paddy fields in Southern China. Therefore, the aim of the current study was to investigate how the soil microbial community structure varies with different fertilization treatments after 31 years of continuous cropping in double-cropped paddy fields in Southern China. The microbial community structure was analysed using the PLFA profiling method.

Materials and methods

Site description

The long-term experiment, established in 1986, is located in Ning Xiang County (28°07′N, 112°18′E, 36 m asl) of Hunan Province, China, under a continental monsoon climate. Annual mean precipitation is 1553 mm and potential evapotranspiration is 1354 mm. The monthly mean temperature is 17.2 °C. Soil texture in the plough layer (0–20 cm) is silt clay loam with 13.7% sand and 57.7% silt. At the beginning of the study, the surface soil characteristics (0–20 cm) were as follows: soil organic carbon (SOC) 29.4 g/kg, total N 2.0 g/kg, available N 144.1 mg/kg, total phosphorous (P) 0.59 g/kg, available P 12.87 mg/kg, total potassium (K) 20.6 g/kg and available K 33.0 mg/kg. There were three crops in each year: barley (*Hordeum vulgare* L.), early rice and late rice (*Oryza sativa* L.). Barley was sown in the middle of November and harvested in early May of the following year. Early rice was then transplanted and harvested in the middle of July. The growing season of late-transplanted rice lasted from late July to the end of October.

Experimental design

The current experiment was conducted in 2015–2016, using the long-term experiment described above. The experiment had five treatments: control (without fertilizer input, CK), chemical fertilizer alone (MF), rice straw residue and chemical fertilizer (RF), low manure rate and chemical fertilizer (LOM), and high manure

rate and chemical fertilizer (HOM). The design ensured all fertilized treatments received the same amount of N, phosphorus pentoxide (P$_2$O$_5$), potassium oxide (K$_2$O) (the amount of N in chemical fertilizer plus that from rice straw residue or manure) during the early and late rice growing season, respectively. The chemical fertilizers included urea, ordinary superphosphate and potassium chloride. Details of the fertilization managements are listed in Table 1. Before barley sowing and transplanting rice seedlings, organic manure and air-dried rice straw residue was spread manually onto the soil surface and incorporated into the soil at a cultivation depth of 20 cm. Then 0.60 of the chemical N fertilizer was applied at soil tillage before sowing of barley and the remaining N fertilizer was applied by top dressing during tillering (50–55 days after sowing, growth stage [GS] 21–29 according to Zadoks *et al.*, 1974). For rice, 0.70 and 0.60 of the chemical N fertilizer was applied at soil tillage before transplanting of early and late rice, respectively, and the remaining N fertilizer was applied by top dressing during rice growth (7–10 days after transplanting). All P and K fertilizers were applied at soil tillage before sowing of barley or transplantation of rice. There were three replications and each plot size was 66.7 m^2 (10 × 6.67 m). The barley variety Ningmai 8 was used in 2015–2016, sown at 250.0 kg/hm^2. The early rice variety Xiangzaoxian 45 and late rice variety Yueyou 9113 were used in 2016. One-month-old rice seedlings were transplanted with a density of 1 50 000 plants/ha and 2–3 plants per hill.

Soil sampling

Soil samples were collected from the plough layer (0–20 cm) at the late rice harvest in 2016. Three soil samples were taken randomly from each plot. The samples from each plot were mixed and transported in a plastic bag to the laboratory on the same day. After removal of the plant straw residue and visible organisms by hand, the soil samples were air-dried at room temperature till the soil moisture (w/w basis) was in the range of 25–30%. Each sample was sieved through a 2 mm sieve, and then divided into two sub-samples. Half of these sub-samples were maintained at −80 °C until phospholipid extraction, and the other half analysed for other soil properties.

Phospholipid fatty acid analysis

Lipid extraction and PLFA analysis were performed using the method described by Bossio *et al.* (1998). Briefly, 2.0 g (dry equivalent weight) was extracted with a chlorofom-methanolcitrate buffer mixture (1:2:0.8) and the phospholipids were separated from other lipids on a silicic acid column. Phospholipids were isolated from the crude lipid extracts by solid phase extraction in silica gel columns, using 19:0 nonadecanoic acid as internal standard. Neutral lipid, glycolipid and phospholipid fractions were eluted by sequential leaching with dichloromethane, acetone and methanol, respectively. The neutral lipid and glycolipid fractions were discarded and the phospholipid fraction retained for methylation (conversion to fatty acid methyl esters or FAMEs) through mild acid methanolysis, i.e. addition of MeOH/H$_2$SO$_4$ (25:1 v/v) and hexane. The FAMEs were analysed by gas chromatography on a GC fitted with a 30 m 5% phenyl and 95% methylpolysiloxane column. The GC running conditions were 80 °C for 1 min, increasing at 20 °C/min to 160 °C, and then increasing at 5 °C/min to the final temperature of 270 °C, which was held for 5 min (Yao *et al.*, 2000). Peaks were identified

Table 1. Nutrient supply from rice straw, chicken manure and chemical fertilizer under different fertilization treatments

Treatments[a]	Early rice			Late rice			Total		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O
CK	0 + 0.0[b]	0 + 0.0	0 + 0.0	0 + 0	0 + 0.0	0 + 0.0	0.0	0.0	0.0
MF	143 + 0.0	54 + 0.0	63 + 0.0	158 + 0.0	43 + 0.0	81 + 0.0	300.0	97.2	144.0
RF[c]	124 + 18.1	50 + 3.6	38 + 24.7	133 + 24.5	38 + 5.4	48 + 32.8	300.0	97.2	144.0
LOM[d]	96 + 46.5	33 + 21.0	34 + 29.4	110 + 47.3	22 + 21.4	51 + 29.9	300.0	97.2	144.0
HOM[e]	50 + 92.9	12 + 42.0	4 + 58.8	63 + 94.5	0.5 + 42.7	21 + 59.8	300.0	97.2	144.0

N, nitrogen; P_2O_5, phosphorus pentoxide; K_2O, potassium oxide.

[a]MF: chemical fertilizer alone; RF: rice straw residues and chemical fertilizer; LOM: 0.30 organic matter and 0.70 chemical fertilizer; HOM: 0.60 organic matter and 0.40 chemical fertilizer; CK: without fertilizer input.

[b]Input from chemical fertilizer + input from rice straw residue or organic manure. The numbers are in kg/hm². The N, P and K content of air-dry early rice straw was 6.5, 1.3 and 8.9 g/kg, N, P and K content of air-dry late rice straw was 6.8, 1.5 and 9.1 g/kg, respectively, and N, P and K content of decomposed chicken manure was 17.7, 8.0 and 11.2 g/kg, respectively.

[c]For the RF treatment, rice straw return rate (air dry) was 2780, 3600 kg/hm² for early and late rice.

[d]For the LOM treatment, chicken manure application rate (decomposed) was 2625.0, 2670.0 kg/hm² for early and late rice.

[e]For the HOM treatment, chicken manure application rate (decomposed) was 5250.0, 5340.0 kg/hm² for early and late rice.

by comparison of retention times to known standards. The abundance of individual PLFAs was expressed as ng/g dry soil as follows (Yao et al., 2000):

$$C_x(\text{ng/g soil}) = \frac{A_x \times c_i[\text{ng}] \times f \times 1000}{A_i \times W[\text{g}] \times M[\text{ng/μmol}]}$$

where C_x = concentration of the PLFAs studied; A_x = peak area of the PLFAs studied; A_i = peak area of the internal standard (19:0 nonadecanoic acid); c_i = absolute amount of internal standard in the vial (ng); f = response factors of different PLFAs (peak area to concentration ratio compared with internal standard; if not known, then 1); W = weight of soil (g); and M = molecular weight of the fatty acid (ng/μmol).

Phospholipid fatty acid community structure analysis

The PLFA patterns of microbial communities in the soil of different fertilization treatments were compared with respect to the species and total amount of PLFAs, the contents of specific PLFAs, e.g. 16:0, 18:1ω9c and 10Me17:0 indicative of bacteria (Hill et al., 2000), and fungi (Kourtev et al., 2002), as well as 10Me16:0 and 16:1ω5c indicative of sulphate-reducing bacteria and actinomycetes (Hill et al., 2000; Kourtev et al., 2002), respectively. The content (μg/g dry weight soil) of PLFAs and richness indices, Shannon indices and McIntosh indices were used for evaluating the diversity of PLFA composition. The indices were calculated according to Schutter and Dick (2000).

Other measurements

Standard techniques were used to measure the soil total C, total N, total P, total K, available N, available P and available K (Olsen and Sommers, 1982). Grain yield was determined from a 1 m² sampling area at late rice harvest and was expressed as rough (unhulled) rice at 14% moisture content.

Statistical analysis

Phospholipid fatty acid data were analysed using principal components analysis (PCA) to elucidate major variation and covariation for individual PLFAs employing varimax rotation. This was performed on $\log_{10}$ transformed mole percentages of individual PLFAs. The PCA scores were subsequently analysed by analysis of variance with correlation matrix using SPSS Version 3.11. Cluster analysis was performed by SPSS software.

Results

Composition and concentrations of phospholipid fatty acids

The soil under each treatment contained various PLFAs composed of saturated, unsaturated, methyl-branched and cyclopropane fatty acids (Table 2). Twenty-one PLFAs with chain lengths ranging from C12 to C20 were identified in different fertilization treatments. Compared with the control (CK, no fertilizer input) treatment, the application of chemical fertilizer alone (MF), rice straw residue and chemical fertilizer (RF), low manure rate and chemical fertilizer (LOM), and high manure rate and chemical fertilizer (HOM) treatments increased the content of total PLFAs significantly ($P < 0.05$). The microbial community containing C12:0 could not be detected, and the C14:0 3OH, aC15:0, iC15:0, C18:0, C18:1ω9, C18:2ω9, 12 in the community were increased significantly ($P < 0.05$), but the C18:1ω9tandc11 in the community was reduced significantly ($P < 0.05$) with MF treatment. The iC15:0 and C18:1ω9tandc11 in the community were reduced significantly ($P < 0.05$) with the RF treatment. The iC15:0 in the community were reduced significantly ($P < 0.05$) by combined application of organic manure with chemical fertilizer (LOM and HOM). Meanwhile, the C18:1ω9tandc11 in the community were reduced significantly ($P < 0.05$) with HOM treatment.

Soil microbial community structure

The microbial community structure expressed as the relative abundance of G^+ bacteria, G^- bacteria and fungi by PLFAs proportion is presented in Table 3. The LOM treatment had the highest content of G^+ bacteria, G^- bacteria PLFAs with 70.46 and 41.18 μg/g, respectively. The MF treatment had the highest value of G^+ bacteria/G^- bacteria PLFAs with 2.02, and the value of G^+ bacteria/G^- bacteria PLFAs with MF treatment was significantly ($P < 0.05$) higher than that of with RF, LOM, HOM and CK treatments. The RF treatment had the highest content

Table 2. Concentrations of PLFAs (ng/g) in different fertilization treatments

PLFAs	Treatments[a]				
	MF	RF	LOM	HOM	CK
C12:0	nd	nd	1.47	nd	1.26
C14:0	3.1 ± 0.09[b]	3.5 ± 0.10	4.8 ± 0.14	3.1 ± 0.09	2.3 ± 0.07
C14:0 2OH	1.5 ± 0.04	1.8 ± 0.05	1.9 ± 0.05	1.8 ± 0.05	1.4 ± 0.04
C14:0 3OH	2.4 ± 0.07	2.8 ± 0.08	2.6 ± 0.08	2.6 ± 0.07	1.9 ± 0.05
C15:0	2.5 ± 0.07	2.7 ± 0.08	2.4 ± 0.07	2.5 ± 0.07	1.9 ± 0.05
iC15:0	20.2 ± 0.27	1.0 ± 0.05	1.1 ± 0.05	1.0 ± 0.03	9.3 ± 0.14
aC15:0	17.4 ± 0.50	26.7 ± 0.77	37.7 ± 1.09	27.3 ± 0.79	13.6 ± 0.39
C16:0	7.8 ± 0.23	9.7 ± 0.28	16.6 ± 0.48	12.8 ± 0.37	5.4 ± 0.16
C16:0 2OH	2.8 ± 0.08	3.1 ± 0.09	4.7 ± 0.14	2.9 ± 0.08	2.6 ± 0.07
iC16:0	7.3 ± 0.21	8.8 ± 0.25	11.3 ± 0.33	7.9 ± 0.23	5.5 ± 0.16
iC16:1 d9	2.6 ± 0.08	3.3 ± 0.09	5.2 ± 0.15	4.2 ± 0.12	2.1 ± 0.06
C17:0	9.3 ± 0.27	11.5 ± 0.33	16.9 ± 0.49	10.2 ± 0.29	7.4 ± 0.21
iC17:0	6.2 ± 0.18	7.3 ± 0.21	11.4 ± 0.33	7.1 ± 0.20	4.5 ± 0.13
iC17:0 d9,10	1.6 ± 0.05	2.3 ± 0.07	2.9 ± 0.08	2.0 ± 0.06	1.2 ± 0.03
C18:0	4.9 ± 0.14	6.6 ± 0.19	5.9 ± 0.17	5.2 ± 0.15	2.1 ± 0.06
C18:1ω9	14.9 ± 0.43	21.7 ± 0.63	25.8 ± 0.75	20.0 ± 0.58	10.4 ± 0.30
C18:1ω9tandc11	2.8 ± 0.08	3.1 ± 0.09	20.2 ± 0.38	3.3 ± 0.09	15.4 ± 0.44
C18:2ω9,12	17.1 ± 0.49	21.7 ± 0.63	11.3 ± 0.33	20.2 ± 0.58	2.9 ± 0.08
cyc19:0	1.7 ± 0.05	2.3 ± 0.07	3.1 ± 0.09	2.4 ± 0.07	1.5 ± 0.04
C19:0c9,10	13.1 ± 0.38	20.3 ± 0.59	15.3 ± 0.44	20.8 ± 0.60	11.2 ± 0.32
C20:0	1.3 ± 0.04	1.6 ± 0.04	2.0 ± 0.06	1.5 ± 0.05	1.4 ± 0.04
Total PLFAs (ng/g)	140.1 ± 4.05	161.5 ± 4.66	203.0 ± 5.86	158.3 ± 4.57	103.67 ± 2.99

nd, Not detected.
[a]MF: chemical fertilizer alone; RF: rice straw residues and chemical fertilizer; LOM: 0.30 organic matter and 0.70 chemical fertilizer; HOM: 0.60 organic matter and 0.40 chemical fertilizer; CK: without fertilizer input. The same as below.
[b]Values are presented as mean ± s.e. (*n* = 3).

Table 3. PLFAs (μg/g) in Gram-positive bacteria (G[+] bacteria), Gram-negative bacteria (G[−] bacteria), fungi and the ratio among them in different fertilization treatments

Items	Treatments[a]				
	MF	RF	LOM	HOM	CK
Gram-positive bacteria (G[+] bacteria)	54 ± 1.6[b]	46 ± 1.3	70 ± 2.0	44 ± 1.3	35 ± 1.0
Gram-negative bacteria (G[−] bacteria)	26.9 ± 0.78	35 ± 1.0	41 ± 1.2	32.7 ± 0.94	20.4 ± 0.59
G[+] bacteria/G[−] bacteria	2.0 ± 0.06	1.3 ± 0.04	1.7 ± 0.05	1.3 ± 0.04	1.7 ± 0.05
Fungi	16.3 ± 0.47	22.7 ± 0.65	13.8 ± 0.40	20.4 ± 0.59	2.5 ± 0.07
Fungi/G[+] bacteria + G[−] bacteria	0.2 ± 0.01	0.3 ± 0.01	0.1 ± 0.01	0.3 ± 0.01	0.1 ± 0.01

[a]MF: chemical fertilizer alone; RF: rice straw residues and chemical fertilizer; LOM: 0.30 organic matter and 0.70 chemical fertilizer; HOM: 0.60 organic matter and 0.40 chemical fertilizer; CK: without fertilizer input. The same as below.
[b]Values are presented as mean ± S.E. (*n* = 3).

of fungi PLFAs (22.68 μg/g), and the value of fungi PLFA content in the RF treatment was significantly ($P < 0.05$) higher than that in the MF, LOM, HOM and CK treatments. The RF and HOM treatments had the highest value of fungi/G[+] bacteria + G[−] bacteria (0.28 and 0.27, respectively), and the value of fungi/G[+] bacteria + G[−] bacteria with RF and HOM treatments was significantly ($P < 0.05$) higher than that of the MF, LOM and CK treatments.

Diversity of soil microbial communities

The HOM treatment had the highest value of richness indices and the value decreased in the order HOM>LOM>RF>CK>MF. The value of Shannon indices for HOM and LOM treatments was significantly ($P < 0.05$) higher than that in the MF and CK treatments, and the value decreased in the order

Table 4. Diversity of microbial communities in soil at late rice harvest under different the fertilization treatments

Treatments[a]	Items		
	Richness indices	Shannon indices	McIntosh indices
MF	15.1 ± 0.44[b]	2.2 ± 0.06	5.9 ± 0.42
RF	15.9 ± 0.46	2.5 ± 0.07	4.8 ± 0.41
LOM	16.2 ± 0.47	2.6 ± 0.07	6.3 ± 0.43
HOM	16.7 ± 0.48	2.7 ± 0.08	6.6 ± 0.45
CK	15.6 ± 0.45	2.4 ± 0.07	5.5 ± 0.44

[a]MF: chemical fertilizer alone; RF: rice straw residues and chemical fertilizer; LOM: 0.30 organic matter and 0.70 chemical fertilizer; HOM: 0.60 organic matter and 0.40 chemical fertilizer; CK: without fertilizer input. The same as below.
[b]Values are presented as mean ± S.E. ($n = 3$).

HOM≈LOM>RF>CK>MF. Meanwhile, the value of McIntosh indices in the HOM treatment was significantly ($P < 0.05$) higher than that of the RF and CK treatments, with the value decreasing in the order HOM>LOM>MF>CK>RF (Table 4).

Soil microbial phospholipid fatty acid and principal component analysis

Principal components analysis also identified fatty acids that were important in explaining the variability in PLFA profiles (Fig. 1). The ordination plot in Fig. 1 illustrates the difference in PLFA composition of the five fertilization treatments, where PC1 and PC2 account for 65.5 and 24.3% of the variation, respectively. In addition to C14:0 3OH and C18:0 included in PC1 and PC2, most of the unsaturated fatty acids and cyclic fatty acids were reflected in PC1 and the fungus and fatty acids were reflected in PC2.

The ordination plot in Fig. 2 illustrates the difference in PLFA composition of the five fertilization treatments, where PC1 and PC2 account for 73.5 and 13.6% of the variation, respectively. The five fertilization treatments are found on the right-hand side of the plot, and the RF, LOM, HOM and CK treatments are found in the first quadrant, whereas the MF treatment are found in the second quadrant. The ordination plot shows that the RF and HOM treatments data formed a cluster, with positive scores for PC1 and PC2, whereas data points for MF treatment clusters were away from the RF, LOM, HOM and CK treatments and had negative scores for PC2. In addition, there are differences in PLFA composition between the LOM treatment and MF, RF, HOM and CK treatments. It is suggested that microbial community composition might be dissimilar under different fertilization treatments except under the MF treatment.

Correlation between microbial characteristics and microbial diversity

The richness and Shannon indices had significant ($P < 0.05$) positive correlation with the content of PLFAs, G^+ bacteria, G^- bacteria and fungi. The value of fungi/G^+ bacteria + G^- bacteria had a significant ($P < 0.05$) positive correlation with the content of PLFAs, G^+ bacteria, G^- bacteria, available N and available K, while the value of fungi/G^+ bacteria + G^- bacteria had significant ($P < 0.01$) positive correlation with fungi, total C, total N and
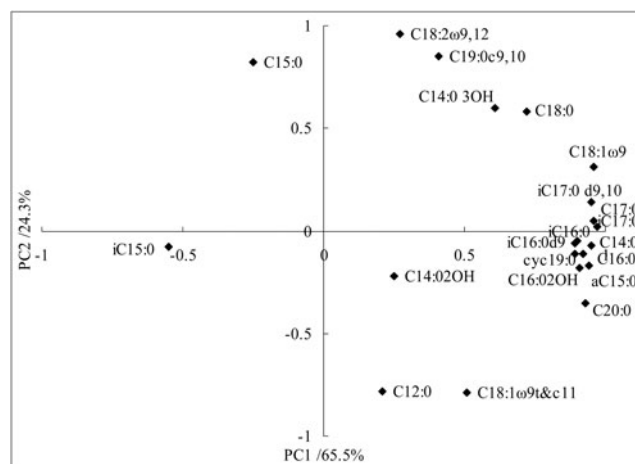
Fig. 1. Principal component analysis showing loading values for individual phospholipid fatty acids.
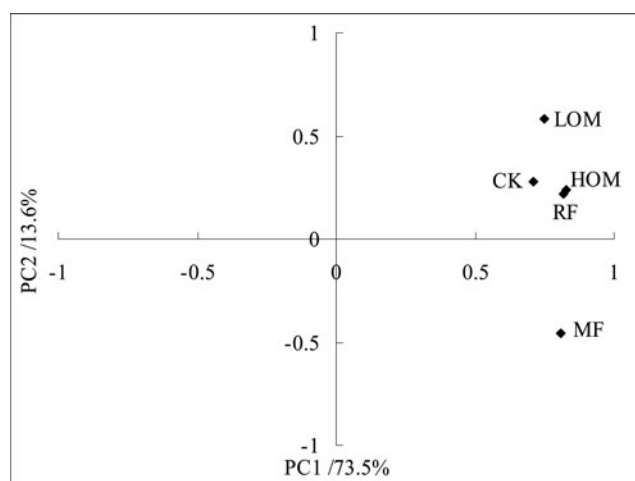
Fig. 2. Principal component analysis showing variations in phospholipid fatty acid patterns with different fertilization treatments. MF: chemical fertilizer alone; RF: rice straw residues and chemical fertilizer; LOM: 0.30 organic matter and 0.70 chemical fertilizer; HOM: 0.60 organic matter and 0.40 chemical fertilizer; CK: without fertilizer input.

grain yield. On the other hand, the value of G^+ bacteria/G^- bacteria had significant ($P < 0.01$) negative correlation with the content of PLFAs, G^+ bacteria, G^- bacteria and fungi. McIntosh indices had significant ($P < 0.05$) positive correlation with the content of G^+ bacteria, G^- bacteria and fungi (Table 5). The richness, Shannon and McIntosh indices were not significantly related to soil nutrient variables and grain yield of rice, whereas the fungi/G^+ bacteria + G^- bacteria had significantly ($P < 0.01$) positive correlation with soil total C, total N and grain yield of rice (Table 5).

Discussion

Soil microbial community structure and fertilization practices

Many studies have demonstrated that agricultural management practices influence soil physical and chemical characteristics, causing changes in the soil microbial community (Kong *et al.*, 2006; Williams *et al.*, 2013). Microbial communities include viruses, eubacteria, archaebacteria, fungi, protozoa, micrometazoa

Table 5. Correlation of soil microbial characteristics, microbial diversity indices to soil nutrients and grain yield of rice

Items	Total PLFAs	G$^+$ bacteria	G$^-$	Fungi	Total C	Total N	Total P	Total K	Available N	Available P	Available K	Grain yield
G$^+$ bacteria/G$^-$ bacteria	−0.72[a]	−0.87[a]	−0.90[a]	−0.66[a]	0.31	0.20	0.05	−0.78[a]	−0.19	−0.18	0.15	−0.38
Fungi/G$^+$ bacteria + G$^-$ bacteria	0.45[a]	0.47[a]	0.48[a]	0.89[a]	0.92[a]	0.91[a]	0.29	−0.02	0.62[a]	−0.05	0.60[a]	0.81[a]
Richness indices	0.58[a]	0.67[a]	0.68[a]	0.81[a]	0.17	0.17	0.57	0.55	0.39	0.40	0.32	0.22
Shannon indices	0.65[a]	0.70[a]	0.72[a]	0.79[a]	0.13	0.13	0.54	0.52	0.34	0.36	0.29	0.37
McIntosh indices	0.43	0.51[a]	0.54[a]	0.56[a]	0.15	0.14	0.52	0.51	0.33	0.38	0.28	0.18

[a]Significant at P 0.01, 0.05, respectively.

and algae (Vestal and White, 1989). Despite their small volume, soil microorganisms are key players in the global cycling of organic matter, recycling organic residues or mineralizing them to CO_2, H_2O, N, P, sulphur (S) and other nutrients (Wieland et al., 2001). The PLFA profile method is acknowledged as a good technique with sufficient resolution to study the changes in microbial community structure in response to environmental perturbations (Yao et al., 2000). In the current study, the soil microbial community under different fertilization practices was investigated using the PLFA method. The results showed that total content of PLFAs was increased significantly by application of straw residue and organic manure (RF, LOM and HOM). Total PLFA biomass in RF, LOM and HOM treatments increased significantly in comparison with the control soil. The reasons for the current results may be that application of straw residue and manure provided a substrate for soil microbial growth (Ai et al., 2012; Zhang et al., 2016). The change in soil pH may also have contributed to the differences observed because soil pH is known as an important factor affecting soil microbial biomass (Aciego Pietri and Brookes, 2009). Similar results were reported by Clegg (2006), who found that long-term manure treatment increased microbial PLFAs. In addition, applications of chemical fertilizer promoted growth of microbial communities that were limited by mineral nutrients, which also caused a shift in the factors that were limiting microbial growth, and resulted in total PLFA biomass under MF treatment lower than that with RF, LOM and HOM treatments.

Data from the current study revealed that branched fatty acids were used as indicators of G$^+$ bacteria biomass, and the contents of fatty acids 18:1ω7, 16:lω7, cy17:0 and cy19:0 were used as indicators of G$^-$ bacteria (Frostegård and Bååth, 1996). In the current study, the PCA indicated that RF and LOM treatments increased the percentage of cyclopropane fatty acids (cy17:0, cy19:0), whereas they decreased the percentage of unsaturated fatty acids; the reason may be that G$^-$ bacteria microbial biomarkers were more reactive to the straw residue and organic manure (Tian et al., 2017). Furthermore, the lower percentage of unsaturated fatty acids with RF and LOM treatments may be related to the low organic manure application rates and the high mineral fertilizer application rates (Dong et al., 2014). For bacteria, the ratio of cy19:0 to 18:1ω7 has been proposed as an indicator of stress conditions, as it increases under situations such as no growth, acidic conditions, low oxygen and high temperature (Guckert et al., 1986). In the current study, a significant decrease in this ratio occurred under the MF treatment compared with RF, LOM and HOM treatments, perhaps because bacteria growth was inhibited by mineral fertilizer and facilitated by straw residue and organic manure, suggesting the bacteria in paddy soils may be related to combined application of straw residue and organic manure with mineral fertilizer. However, the cause leading to this decrease requires further research.

In the current study, some soil microbial community characteristics were responsive to fertilization practices. The fungi/G$^+$ bacteria + G$^-$ bacteria had a significant, positive correlation with soil total C, total N and grain yield of rice. It might be presumed that microbial community structure was affected by fertilization practices (Yu et al., 2013; Tang et al., 2016). The nature of nutrient inputs affects the availability of soil nutrients and substrates as well as the growth of rice plants, affecting the characteristics of microbial habitats in soil (Ai et al., 2012; Williams et al., 2013; Li et al., 2015). Zhang et al. (2014) also reported that fungi were positively correlated with soil organic C, possibly because

fungi were distributed mainly in macroaggregates. Interestingly, the soil microbial diversity indices calculated based on PLFA community fingerprinting technique in the current study did not correlate significantly with the measured soil nutrient variables and grain yield of rice. This may be partly because of the limitations with respect to generating diversity information by the PLFA technique, such as difficulty in linking signature molecules with specific groups of microorganisms, and in not being able to reveal information at a species level (Hill *et al*., 2000).

Microbial characteristics and fertilization practices

In nature, microbial communities play an important role in the biosphere, primarily in recycling biologically important elements (Wieland *et al*., 2001). High proportions of G^+ bacteria, relatively to G^- bacteria, are usually associated with soils low in SOC such as arable soils (Herman *et al*., 2012). Therefore, G^+ bacteria, G^- bacteria and fungi were detected in paddy soils in the current experiment using PLFAs as specific biomarkers. In the current study, the results indicated that G^+ bacteria, G^- bacteria PLFAs with LOM treatment were higher than that of other treatments. This may be because low organic manure application rates and high mineral fertilizer application rates provide a large amount of readily available substrates for sustained microbial biomass for a long period and organic manure has a significant effect on the content of G^+ and G^- bacteria (Rinnan *et al*., 2008). The MF treatment had the highest value of G^+ bacteria/G^- bacteria PLFAs: possible reasons for this include: (1) the fungal PLFA C18:2ω9,12 was detected, but many kinds of fungi from the soil were not really measured (Calderón *et al*., 2001); (2) it is probably related to soil pH (Zhang *et al*., 2016). However, the value of G^+ bacteria/G^- bacteria PLFAs was decreased by application of organic fertilization, possibly because G^- bacteria, which are sensitive to copiotrophic conditions (Kieft *et al*., 1994), are often stimulated by added straw residue and organic matter, resulting in a low ratio of G^+ bacteria/G^- bacteria (Larkin *et al*., 2006).

The value of soil fungi/bacteria was used as a measure of their responses to environmental change and changes in soil microbial communities (Strickland and Rousk, 2010). Some studies have found that a higher value of soil fungi/bacteria was indicative of a sustainable agro-ecosystem with lower environmental impact (De Vries *et al*., 2006; Sun *et al*., 2016). In the current study, it was indicated that the content of fungi PLFAs and value of fungi/G^+ bacteria + G^- bacteria were higher in the RF treatment than the other treatments, indicating that combined application of straw residue with chemical fertilizer also provides a large amount of readily available substrate for sustained microbial biomass over a long period, and that straw residue with high C/N ratio stimulates fungal growth and thus increases the value of fungi/G^+ bacteria + G^- bacteria (Dong *et al*., 2014). It has been suggested that application of straw residue is a better way to improve the sustainable ecosystem of paddy fields with improved tolerance to environmental impact.

In addition, there were significant correlations between soil microbial characteristics and microbial diversity. These results suggest that soil microbial community is strongly driven by the different type of fertilizers (Yu *et al*., 2013). Meanwhile, the richness, Shannon and McIntosh indices were the highest with organic manure practices (LOM and HOM). Reasons for this may include: (1) the addition of nutrients in the field allows better growth of soil microbes (Williams *et al*., 2013; Li *et al*., 2015); (2) higher extracellular enzyme activities in the rhizosphere contribute to maintenance of high microbial diversity in the soils (Ai *et al*., 2012), in agreement with the results of Yu *et al*. (2013).

Conclusions

Long-term fertilization practices have a great impact on the soil microbial community. The current results showed that the content of total PLFAs, fungal PLFAs and value of fungi/G^+ bacteria + G^- bacteria were increased by long-term combined application of straw residue and organic manure with chemical fertilizer. The proportion of G^+ bacteria was higher than that of G^- bacteria in different fertilization practices soils. Principal component analyses showed that the content of most PLFA biomarkers increased in response to the RF, LOM and HOM treatments, which indicates that the diversity of microbial communities in paddy soils was increased by combined application of straw residue and organic manure with chemical fertilizer. As a result, it is beneficial to improve the soil microbial community structure in double-cropped paddy fields in Southern China by long-term combined application of straw residue or organic manure with chemical fertilizer practices.

References

Aciego Pietri JC and Brookes PC (2009) Substrate inputs and pH as factors controlling microbial biomass, activity and community structure in an arable soil. *Soil Biology and Biochemistry* **41**, 1396–1405.

Ai C, Liang GQ, Sun JW, Wang XB and Zhou W (2012) Responses of extracellular enzyme activities and microbial community in both the rhizosphere and bulksoil to long-term fertilization practices in a fluvo-aquic soil. *Geoderma* **173–174**, 330–338.

Bossio DA, Scow KM, Gunapala N and Graham KJ (1998) Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. *Microbial Ecology* **36**, 1–12.

Bowles TM, Acosta-Martinez V, Calderon F and Jackson LE (2014) Soil enzyme activities, microbial communities, and carbon and nitrogen availability in organic agroecosystems across an intensively-managed agricultural landscape. *Soil Biology and Biochemistry* **68**, 252–262.

Calderón FJ, Jackson LE, Scow KM and Rolston DE (2001) Short-term dynamics of nitrogen, microbial activity, and phospholipid fatty acids after tillage. *Soil Science Society of America Journal* **65**, 118–126.

Chodak M and Niklińska M (2010) Effect of texture and tree species on microbial properties of mine soils. *Applied Soil Ecology* **46**, 268–275.

Clegg CD (2006) Impact of cattle grazing and inorganic fertilizer additions to managed grasslands on the microbial community composition of soils. *Applied Soil Ecology* **31**, 73–82.

Demoling F, Nilsson LO and Bååth E (2008) Bacterial and fungal response to nitrogen fertilization in three coniferous forest soils. *Soil Biology and Biochemistry* **40**, 370–379.

De Vries FT, Hoffland E, van Eekeren N, Brussaard L and Bloem J (2006) Fungal/bacterial ratios in grasslands with contrasting nitrogen management. *Soil Biology and Biochemistry* **38**, 2092–2103.

Dong WY, Zhang XY, Dai XQ, Fu XL, Yang FT, Liu XY, Sun XM, Wen XF and Schaeffer S (2014) Changes in soil microbial community composition in response to fertilization of paddy soils in subtropical China. *Applied Soil Ecology* **84**, 140–147.

Frostegård A and Bååth E (1996) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biology and Fertility of Soils* **22**, 59–65.

García-Orenes F, Morugan-Coronado A, Zornoza R and Scow K (2013) Changes in soil microbial community structure influenced by agricultural management practices in a Mediterranean agro-ecosystem. *PLoS ONE* **8**, e80522.

Guckert JB, Hood MA and White DC (1986) Phospholipid, ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*: increases in the *trans/cis* ratio and proportions of cyclopropyl fatty acids. *Applied and Environmental Microbiology* **52**, 794–801.

Hao XH, Liu SL, Wu JS, Hu RG, Tong CL and Su YY (2008) Effect of long-term application of inorganic fertilizer and organic amendments on soil organic matter and microbial biomass in three subtropical paddy soils. *Nutrient Cycling in Agroecosystems* **81**, 17–24.

Herman DJ, Firestone MK, Nuccio E and Hodge A (2012) Interactions between an arbuscular mycorrhizal fungus and a soil microbial community mediating litter decomposition. *FEMS Microbiology Ecology* **80**, 236–247.

Hill GT, Mitkowski NA, Aldrich-Wolfe L, Emele LR, Jurkonie DD, Ficke A, Maldonado-Ramirez S, Lynch ST and Nelson EB (2000) Methods for assessing the composition and diversity of soil microbial communities. *Applied Soil Ecology* **15**, 25–36.

Hu XJ, Liu JJ, Wei D, Zhu P, Cui XA, Zhou BK, Chen XL, Jin J, Liu XB and Wang GH (2017) Effects of over 30-year of different fertilization regimes on fungal community compositions in the black soils of northeast China. *Agriculture, Ecosystems and Environment* **248**, 113–122.

Islam MR, Chauhan PS, Kim Y, Kim M and Sa T (2011) Community level functional diversity and enzyme activities in paddy soils under different long-term fertilizer management practices. *Biology and Fertility of Soils* **47**, 599–604.

Kieft T, Ringelberg D and White D (1994) Changes in ester-linked phospholipid fatty acid profiles of subsurface bacteria during starvation and desiccation in a porous medium. *Applied and Environmental Microbiology* **60**, 3292–3299.

Kong XB, Zhang FR, Wei Q, Xu Y and Hui JG (2006) Influence of land use change on soil nutrients in an intensive agricultural region of North China. *Soil and Tillage Research* **88**, 85–94.

Kourtev PS, Ehrenfeld JG and Häggblom MH (2002) Exotic plant species alter the microbial community structure and function in the soil. *Ecology* **83**, 3152–3166.

Larkin RP, Honeycutt CW and Griffin TS (2006) Effect of swine and dairy manure amendments on microbial communities in three soils as influenced by environmental conditions. *Biology and Fertility of Soils* **43**, 51–61.

Li J, Cooper JM, Lin ZA, Li YT, Yang XD and Zhao BQ (2015) Soil microbial community structure and function are significantly affected by long-term organic and mineral fertilization regimes in the North China Plain. *Applied Soil Ecology* **96**, 75–87.

Moche M, Gutknecht J, Schulz E, Langer U and Rinklebe J (2015) Monthly dynamics of microbial community structure and their controlling factors in three floodplain soils. *Soil Biology and Biochemistry* **90**, 169–178.

Olsen SR and Sommers LE (1982) Phosphorus. In Page AL, Miller RH and Keeney DR (eds), *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*, 2nd Edn. Madison, WI, USA: American Society of Agronomy and Soil Science of America, pp. 403–430.

Rinnan R, Michelsen A and Jonasson S (2008) Effects of litter addition and warming on soil carbon, nutrient pools and microbial communities in a subarctic heath ecosystem. *Applied Soil Ecology* **39**, 271–281.

Schutter ME and Dick RP (2000) Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities. *Soil Science Society of America Journal* **64**, 1659–1668.

Strickland MS and Rousk J (2010) Considering fungal: bacterial dominance in soils-methods, controls, and ecosystem implications. *Soil Biology and Biochemistry* **42**, 1385–1395.

Sun R, Dsouza M, Gilbert JA, Guo X, Wang D, Guo Z, Ni Y and Chu H (2016) Fungal community composition in soils subjected to long-term chemical fertilization is most influenced by the type of organic matter. *Environmental Microbiology* **18**, 5137–5150.

Tang HM, Xiao XP, Wang K, Li WY, Liu J and Sun JM (2016) Methane and nitrous oxide emissions as affected by long-term fertilizer management from double-cropping paddy fields in Southern China. *Journal of Agricultural Science, Cambridge* **154**, 1378–1391.

Tian J, Lou YL, Gao Y, Fang HJ, Liu ST, Xu MG, Blagodatskaya E and Kuzyakov Y (2017) Response of soil organic matter fractions and composition of microbial community to long-term organic and mineral fertilization. *Biology and Fertility of Soils* **53**, 523–532.

Vestal JR and White DC (1989) Lipid analysis in microbial ecology: quantitative approaches to the study of microbial communities. *Bioscience* **39**, 535–541.

Wang H, Yang JP, Yang SH, Yang ZC and Lv YM (2014) Effect of a 10 °C-elevated temperature under different water contents on the microbial community in a tea orchard soil. *European Journal of Soil Biology* **62**, 113–120.

White DC, Davis WM, Nickels JS, King JD and Bobbie RJ (1979) Determination of the sedimentary microbial biomass by extractable lipid phosphate. *Oecologia* **40**, 51–62.

Wieland G, Neumann R and Backhaus H (2001) Variation of microbial communities in soil, rhizosphere, and rhizoplane in response to crop species, soil type, and crop development. *Applied and Environmental Microbiology* **67**, 5849–5854.

Williams A, Börjesson G and Hedlund K (2013) The effects of 55 years of different inorganic fertiliser regimes on soil properties and microbial community composition. *Soil Biology and Biochemistry* **67**, 41–46.

Yao H, He Z, Wilson MJ and Campbell CD (2000) Microbial biomass and community structure in a sequence of soils with increasing fertility and changing land use. *Microbial Ecology* **40**, 223–237.

Yevdokimov IV, Gattinger A, Buegger F, Schloter M and Munch JC (2012) Changes in the structure and activity of a soil microbial community caused by inorganic nitrogen fertilization. *Microbiology (Reading, England)* **81**, 743–749.

Yu WT, Bi ML, Xu YG, Zhou H, Ma Q and Jiang CM (2013) Microbial biomass and community composition in a Luvisol soil as influenced by long-term land use and fertilization. *Catena* **107**, 89–95.

Zadoks JC, Chang TT and Konzak CF (1974) A decimal code for the growth stages of cereals. *Weed Research* **14**, 415–421.

Zhang H, Ding W, He X, Yu H, Fan J and Liu D (2014) Influence of 20-year organic and inorganic fertilization on organic carbon accumulation and microbial community structure of aggregates in an intensively cultivated sandy loam soil. *PLoS ONE* **9**, e92733.

Zhang B, Gao Q, Xu S, Ma L and Tian C (2016) Long-term effect of residue return and fertilization on microbial biomass and community composition of a clay loam soil. *Journal of Agricultural Science, Cambridge* **154**, 1051–1061.

Zhong W, Gu T, Wang W, Zhang B, Lin X, Huang Q and Shen W (2010) The effects of mineral fertilizer and organic manure on soil microbial community and diversity. *Plant and Soil* **326**, 511–522.

Zhou J, Jiang X, Zhou B, Zhao B, Ma M, Guan D, Li J, Chen S, Cao F, Shen D and Qin J (2016) Thirty four years of nitrogen fertilization decreases fungal diversity and alters fungal community composition in black soil in northeast China. *Soil Biology and Biochemistry* **95**, 135–143.