Plant Soil (2010) 326:511–522
DOI 10.1007/s11104-009-9988-y

REGULAR ARTICLE

The effects of mineral fertilizer and organic manure on soil microbial community and diversity

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Received: 8 December 2008 / Accepted: 25 March 2009 / Published online: 23 April 2009

Abstract The effects of mineral fertilizer (NPK) and organic manure on phospholipid fatty acid profiles and microbial functional diversity were investigated in a long-term (21-year) fertilizer experiment. The experiment included nine treatments: organic manure (OM), organic manure plus fertilizer NPK (OM + NPK), fertilizer NPK (NPK), fertilizer NP (NP), fertilizer NK (NK), fertilizer N (N), fertilizer P (P), fertilizer K (K), and the control (CK, without fertilization). The original soil was extremely eroded, characterized by low pH and deficiencies of nutrients, particularly N and P. The application of OM and OM + NPK greatly increased crop yields, soil pH, organic C, total N, P and K, available N, P and K content. Crop yields, soil pH, organic C, total N and available N were also clearly increased by the application of mineral NPK fertilizer. The amounts of total PLFAs, bacterial, Gram-negative and actinobacterial PLFAs were highest in the OM + NPK treatment, followed by the OM treatment, whilst least in the N treatment. The amounts of Gram-positive and anaerobic PLFAs were highest in the OM treatment whilst least in the P treatment and the control, respectively. The amounts of aerobic and fungal PLFAs were highest in the NPK treatment whilst least in the N and P treatment, respectively. The average well color development (AWCD) was significantly increased by the application of OM and OM + NPK, and the functional diversity indices including Shannon index (H'), Simpson index (D) and McIntosh index (U) were also significantly increased by the application of OM and OM + NPK. Principal component analysis (PCA) of PLFA profiles and C source utilization patterns were used to describe changes in microbial biomass and metabolic fingerprints from nine fertilizer treatments. The PLFA profiles from OM, OM + NPK, NP and NPK were significantly different from that of CK, N, P, K and NK, and C source utilization patterns from OM and OM + NPK were clearly different from organic manure deficient treatments (CK, N, P, K, NP, NK 6

Responsible editor: Petra Marschner.

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and NPK). Stepwise multiple regression analysis showed that total N, available P and soil pH significantly affected PLFA profiles and microbial functional diversity. Our results could provide a better understanding of the importance of organic manure plus balanced fertilization with N, P and K in promoting the soil microbial biomass, activity and diversity and thus enhancing crop growth and production.

Keywords CLPP · Long-term fertilization · Microbial biomass · PLFA · Red soil · Soil fertility

Introduction

The influence of soil microorganisms on soil quality has been emphasized more recently (Visser and Parkinson 1992; Mele and Crowley 2008). Understanding soil microbial ecology is increasingly recognized as important for the restoration and sustainability of ecosystems (Steenwerth et al. 2002; Potthoff et al. 2006). Soil microorganisms are critical to the maintenance of soil function because of their contributions to soil structure formation; decomposition of organic matter; toxin removal; and the biogeochemical cycling of carbon, nitrogen, phosphorus, and sulphur (Karlen et al. 1997; Doran and Zeiss 2000; Paul 2007). The extent of soil microbial diversity in agricultural soils is also critical to the maintenance of soil health and quality (Garbeva et al. 2004; Shen et al. 2008). Since the growth and activity of microorganisms are functions of soil properties, such as nutrition, texture, pH, temperature, soil water content, etc., and they are sensitive indicators of changes in soil properties (Mele and Crowley 2008). A variety of microbial parameters have the potential for use as diagnostic indicators of soil quality such as microbial biomass and microbial diversity (Kennedy and Smith 1995; Sparling 1997; Anderson 2003; Bending et al. 2004).

Many studies have focused on the influence of long-term fertilizer applications on soil physical properties (Pernes-Debuyers and Tessier 2004), soil fertility (Mallarino and Borges 2006), soil organic matter, and crop yield (Belay et al. 2002; Cai and Qin 2006). Mineral fertilizer and organic manure maintain or improve crop yields, and induce changes in soil chemical, physical and biological properties. These changes, in the long-term, are believed to have significant influences on the quality and productive capacity of the soil (Acton and Gregorich 1995; Belay et al. 2002; Zhong and Cai 2007). However, available information is conflicting and uncertainties still remain about the long-term influence of mineral fertilizer and organic manure on soil microbial biomass and microbial diversity. Several studies documented that mineral fertilizers or organic manure increased microbial biomass and microbial diversity (Belay et al. 2002; Chu et al. 2007; Zhong and Cai 2007; He et al. 2008), but Sarathchandra et al. (2001) reported that nitrogen and phosphate fertilizers had no significant effects on soil microbiological properties in pasture soils. For example, long-term application of N fertilizers lead to soil ammonia-oxidizing bacteria community shifts and increased soil nitrification potential (Chu et al. 2007). In contrast, He et al. (2007) observed highest numbers of ammonia-oxidizing bacteria and ammonia-oxidizing archaea in the long-term organic manure plus mineral NPK fertilizer treatment whilst least numbers in the N treatment due to soil acidification (McAndrew and Malhi 1992; Pernes-Debuyers and Tessier 2004; He et al. 2007).

Red soils (Ultisols and Oxisols in US Soil Taxonomy), one of the typical subtropical soils of China, are widely distributed in southeast of China (on the north bound by the Yangtze River and on the west bound by the Yun-Gui Plateau). Red soils cover about 1.13 million km^2 or 11.8% of the country's land surface, and support 22.5% of the nation's population (Zhao 2002). Red soils are heavily weathering and leaching soils, and are characterized by low pH and deficiencies in available nutrients, particularly N and P (Zhong and Cai 2007; He et al. 2008). Due to deforestation and intensive use, 24.8 million hectare of these red soils have become eroded (Zhao 2002). Consequently, most fields have had low organic carbon content and low crop productivity (Lou et al. 2004; Zhong and Cai 2007). Maize planting is one of the main utilization manners of red soils. Crop growth is usually restricted by low pH and deficiencies in nutrition provision. Great efforts have been made since the mid-1980s to restore these degraded red soils, and a number of long-term experiments were set up at that time (Zhao 2002). So far, little information is available on the changes in microbial parameters during the restoration of degraded soils (Zhong and Cai 2007; Zhong et al. 2007; He et al. 2008). Long-term effects of inorganic fertilizers on biochemical properties, microbial biomass and community functional diversity in a paddy soil derived from

quaternary red clay were documented (Zhong and Cai 2007; Zhong et al. 2007). Most microbial parameters were mainly correlated with soil organic carbon content rather than P and N, indicating that the application of P and N did not directly affect microbial parameters in the soil, but did so indirectly by increasing crop yields, thus promoting the accumulation of soil organic matter (Zhong and Cai 2007). However, little is known about soil microbial biomass, activity and diversity under long-term mineral fertilizer and organic manure in the upland red soil. For our study we selected one long-term field experiment which was set up in Jiangxi Institute of Red Soil, Jiangxi Province, China in 1986 to determine soil microbial parameters under various treatments. The field site is located in the typical red earth hilly region in the mid-subtropical monsoon landscape zone of South China.

By examining the PLFA profiles and C source utilization patterns in a long-term fertilizer experiment that has been carried out since 1986, the aim of this study was to investigate the effects of mineral fertilizer and organic manure on soil microbial biomass, activity and diversity, and find out the main factor(s) that influence these microbial parameters.

Materials and methods

Site description and soil sampling

The long-term field fertilizer experiment was carried out in Jiangxi Institute of Red Soil (116°20′24″N, 28° 15′30″E), Jiangxi Province, China in 1986. This region has a typical subtropical monsoon climate with an annual precipitation of 1537 mm, annual evaporation of 1100–1200 mm, and a mean annual temperature of 17.5°C. Before the experiment was set up in an uncultivated wasteland with the soil derived from quaternary red clay, the surface soil was completely eroded and soil organic matter content was very low. The soil had a pH (H_2O) of 5.36, contained 8.59 g kg^{-1} of organic C, 1.00 g kg^{-1}, 0.57 g kg^{-1} and 15.70 g kg^{-1} of total N, P and K, 74.87, 12.75 and 91.00 mg kg^{-1} of available N, P and K, respectively, at the beginning of the experiment in 1986. When the experiment was set up, the field was leveled off and dried for double cropping of maize (*Zea mays* L.) crops (i.e. early and late maize crops) from early April to the end of November and was in fallow for the rest time period. The experiment consisted of nine fertilizer treatments with three replicates arranged in a completely randomized design: 1) the control (CK, without fertilization), 2) mineral N fertilizer (N), 3) mineral P fertilizer (P), 4) mineral K fertilizer (K), 5) mineral NP fertilizer (NP), 6) mineral NK fertilizer (NK), 7) mineral NPK fertilizer (NPK), 8) organic manure (OM) and 9) organic manure plus mineral NPK fertilizer (OM + NPK). N, P, K and organic manure were applied in the form of urea (120 kg N ha^{-1} per year), calcium superphosphate (60 kg P_2O_5 ha^{-1} per year), KCl (120 kg K_2O ha^{-1} per year), and composted pig manure (2,000 kg ha^{-1} per year), respectively. Mineral fertilizers and organic manure were applied as basal fertilization before planting maize.

Soil samples were taken from the plough layer (0–20 cm depth) at seventeen randomly selected points in each plot in March 2007 and mixed to yield a composite sample. Fresh soils were stored at 4°C within one week for microbiological and mineral N analyses. Air dried and sieved (<2 mm) soils were for chemical analyses.

Soil chemical analyses

Soil pH was determined with a glass electrode (soil: water=1:2.5). Soil organic C was determined by the dichromate oxidation and total N by the Kjeldahl digestion. Mineral N was extracted with 2 M KCl (soil:KCl=1:4) for 1 h. Available N in the extracts were determined by an automated procedure (Skalar SAN^{plus} Segmented Flow Analyzer, Skalar Analytic B.V., De Breda, The Netherlands). Total P and available P were extracted with HF-HNO_3-$HClO_4$ and sodium bicarbonate, respectively, and then determined by the molybdenum-blue method. Total K and available K were extracted with HF-HNO_3-$HClO_4$ and ammonium acetate, respectively, and then determined by a flame photometry.

PLFAs analysis

PLFAs were extracted using a modified Bligh-Dyer technique (Bligh and Dyer 1959; Bossio et al. 1998) as described by Brant et al. (2006). Briefly, soils (2 g dry weight) were incubated in a 2:1:0.8 solution of methanol, chloroform, and phosphate buffer. The soil extracts were filtered and the chloroform phases

collected. Phospholipids were separated from glycolipids and neutral lipids using silicic acid bonded solid-phase-extraction columns. Phospholipids were saponified and methylated to fatty-acid methyl esters (FAME). FAME was analyzed using the MIDI Sherlock Microbial Identification System (MIDI, Newark, DE, USA) according to the manufacturer's instructions. Peaks were identified based on comparing retention times with known standards. Concentration of each PLFA was obtained by comparing peak areas with a 19:0 FAME internal standard.

Diagnostic groups of fatty acids were used to calculate bacterial (include Gram-positive, Gram-negative, Aerobes, Anaerobes), fungal and actinobacterial PLFAs (Zelles et al. 1995; Kourtev et al. 2002; Kourtev et al. 2003). PLFAs that contributed less than 1% of the total amount extracted from each sample, or PLFAs that were observed in only one sample were eliminated from the data set, yielding 29 PLFAs for statistical analysis. The PLFA data (29 distinct PLFAs identified) were subjected to principal components analysis (PCA) to examine the variation in the PLFA composition after long-term application of mineral fertilizer and organic manure.

BIOLOG analyses

Community level physiological profiles (CLPP) of the bacterial communities in soils were assessed as described previously (Garland and Mills 1991; Campbell et al. 1997). Three replicates of 10 g composite soils from each fertilizer treatment were suspended in 100 ml of sterile phosphate buffer (0.05 M, pH 7.0) and shaken for 30 min. One ml of this soil suspension was used for serial tenfold dilutions in sterile phosphate buffer (0.05 M, pH 7.0). 150 µl of the 10^{-2} dilution was added to each well of a BIOLOG Eco Micro plate (Biolog, Hayward, CA, USA). Plates were incubated at 25°C, and measured at 590 nm by an Emax precision micro plate reader (Biolog, Hayward, CA, USA). The readings at 96 h incubation were collected by Microlog Rel. 4.2 software (Biolog, Hayward, CA, USA). The data were expressed as the following five parameters (Garland and Mills 1991; Zak et al. 1994): (1) Average well color development (AWCD) for metabolic activity of soil bacterial communities; (2) Shannon index (H'), (3) Simpson index (D) and (4) McIntosh index (U) for the species richness of bacterial community, the most common species in community and the species evenness of community, as respectively sensitive indicators, were calculated as follow:

$$H' = -\sum Pi \cdot \ln(Pi)$$

$$D = 1 - \sum (Pi)^2$$

$$U = \sqrt{\left(\sum ni^2\right)}$$

Where Pi is the ratio of activities on each substrate to the sum of activities on all substrates, ni is activities on each substrate; and (5) Principal component analysis (PCA), which is the most commonly used ordination technique for BIOLOG data analysis, was calculated by SPSS 13.0 for Windows.

Statistics

Significant differences of means in all treatments were judged by Duncan multiple comparison tests at the 5% level with SPSS 13.0 for Windows. To determine the key factor(s) affecting microbial parameters and the quantitative relationships between them, stepwise multiple regression analysis was applied using the criteria of probability of $p<0.05$ to accept and $p>0.1$ to remove a variable from the analysis.

Results

Crop yields, soil pH and nutrient contents

Four-year maize yields in the treatments of the long-term experiment were presented from 2004 to 2007 (Table 1). The maize yields were greatly increased by the application of organic manure. The maize yields were also significantly increased by the application of mineral fertilizer, except in the case of N, P and K treatments. The maize yields were highest in the OM + NPK treatment, followed by the OM and NPK treatments, respectively.

Soil pH, organic C, total N, P and K, available N, P and K content were significantly increased by the application of organic manure (OM and OM + NPK) for 21 years since 1986 (Table 2). Soil pH, organic C, total N and available N were also significantly increased

Table 1 Four-year maize yields (kg ha^{-1}) in the treatments of the long-term experiment

Treatments	Year 2004		Year 2005		Year 2006		Year 2007	
	Early maize	Late maize	Early maize	Late maize	Early maize	Late maize	Early maize	Late maize
CK	450±45 g	750±52 g	900±119 g	990±45 f	185±16 e	533±104 e	810±45 f	953±72 e
N	1680±182 f	1200±248 f	2325±231 e	915±26 f	225±23 e	555±106 e	1575±162 e	1095±183 e
P	480±52 g	375±52 h	1800±90 f	1515±145 e	270±78 e	630±45 e	540±90 f	563±98 f
K	525±69 g	540±119 gh	2010±104 f	1575±135 e	270±45 e	675±0 e	555±52 f	593±130 f
NP	2505±408 e	2130±52 e	3300±94 d	2190±137 d	1320±203 cd	1193±203 d	3015±225 d	2310±364 d
NK	3030±104 d	3030±293 d	3765±213 c	3240±78 c	1185±203 d	1440±119 d	3360±187 c	3023±111 c
NPK	3795±468 c	3435±300 c	4200±231 b	3630±69 b	1463±200 c	1845±162 c	3435±213 c	3240±148 c
OM	4786±418 b	4020±319 b	4065±158 bc	3480±408 bc	1703±136 b	2400±344 b	5161±94 b	3885±175 b
OM + NPK	6451±300 a	5701±26 a	6196±264 a	5821±182 a	3368±113 a	2933±115 a	6586±227 a	4328±209 a

CK, without fertilization; N, mineral N fertilizer; P, mineral P fertilizer; K, mineral K fertilizer; NP, mineral NP fertilizer; NK, mineral NK fertilizer; NPK, mineral NPK fertilizer; OM, organic manure; OM + NPK, organic manure plus mineral NPK fertilizer. Values (Means ± SD, n=3) followed by the same letter are not significantly different within columns (P=0.05).

by the application of mineral NPK fertilizer. Total P and K, and available P and K in soil were significantly increased due to the application of P and K fertilizers (P, NP, K and NK), respectively.

PLFA profiles

A total of 29 PLFAs were identified in the different soil treatments, and they were used for data analysis. PLFA profiles were dominated by the fatty acids cy17:0, 16:0, 2- and 3-OH-FAs, 18:1 ω9, 18:2 ω6,9 and a17:0, which together accounted for approximately 40% of the total PLFAs for all treatments. In contrast, the fatty acid 15:0 was only found in the OM + NPK treatment. The total PLFA biomass is an indicator of the total microbial biomass. The amounts of total PLFAs, bacterial, Gram-negative and actinobacterial PLFAs were found to be highest in the OM + NPK treatment, followed by the OM treatment, whilst least in the N treatment (Table 3). The amounts of Gram-positive and anaerobic PLFAs were highest in the OM treatment whilst least in the P treatment and the control, respectively. The amounts of aerobic and fungal PLFAs were highest in the NPK treatment whilst least in the N and P treatment, respectively.

The PCA plots of the first two principal components (PCs) accounted for 33.7% and 20.2% of the overall variance, and the PLFA profiles showed a

Table 2 Soil pH and nutrient contents after long-term application of mineral fertilizer and organic manure

Treatments	pH (H_2O)	Organic C (g kg^{-1})	Total N (g kg^{-1})	Available N (mg kg^{-1})	Total P (g kg^{-1})	Available P (mg kg^{-1})	Total K (g kg^{-1})	Available K (mg kg^{-1})
CK	5.38±0.06 c	10.34±0.55 de	1.04±0.02 e	51.30±0.92 ef	0.44±0.01 e	0.72±0.80 e	7.62±0.12 d	70.98±2.10 e
N	5.34±0.06 cd	11.03±0.04 bc	1.05±0.01 de	59.06±0.42 c	0.40±0.01 e	0.57±0.05 e	8.46±0.12 c	90.90±5.16 c
P	5.30±0.02 cd	9.99±0.11 e	0.97±0.01 f	51.09±1.38 ef	0.65±0.03 c	4.46±0.03 b	7.94±0.12 d	80.28±1.24 d
K	5.25±0.09 d	10.10±0.15 e	0.95±0.02 f	49.55±1.90 f	0.51±0.01 c	0.73±0.10 e	16.56±0.06 a	131.53±3.35 b
NP	5.51±0.08 b	11.23±0.03 bc	1.17±0.02 c	59.42±0.67 c	0.73±0.04 b	4.49±0.15 b	8.52±0.10 c	84.93±2.31 d
NK	5.32±0.05 cd	10.76±0.07 cd	1.05±0.02 de	53.19±1.84 de	0.46±0.03 de	0.80±0.05 e	13.22±0.17 b	133.16±2.37 b
NPK	5.79±0.10 a	11.33±0.33 b	1.27±0.03 b	62.42±1.37 b	0.69±0.02 bc	3.90±0.07 c	13.06±0.21 b	128.36±1.59 b
OM	5.83±0.09 a	11.36±0.09 b	1.25±0.03 b	63.89±1.39 b	1.08±0.05 a	6.58±0.10 a	12.88±0.10 b	131.47±1.29 b
OM + NPK	5.85±0.06 a	12.42±0.51 a	1.36±0.01 a	69.35±1.25 a	1.14±0.07 a	6.80±0.18 a	16.16±0.65 a	173.29±1.31 a

CK, without fertilization; N, mineral N fertilizer; P, mineral P fertilizer; K, mineral K fertilizer; NP, mineral NP fertilizer; NK, mineral NK fertilizer; NPK, mineral NPK fertilizer; OM, organic manure; OM + NPK, organic manure plus mineral NPK fertilizer. Values (Means ± SD, n=3) followed by the same letter are not significantly different within columns (P=0.05).

Table 3 The amounts of total PLFAs, bacterial, Gram-positive bacterial, Gram-negative bacterial, aerobic, anaerobic, fungal and actinobacterial PLFAs (nmol g^{-1} DW) after long-term application of mineral fertilizer and organic manure

Treatments	Total PLFA	Bacterial PLFA	Gram-positive bacterial PLFA	Gram-negative bacterial PLFA	Aerobic PLFA	Anaerobic PLFA	Fungal PLFA	Actinobacterial PLFA
CK	198.15±50.80 ab	87.58±20.94 cd	21.78±6.75 c	65.81±14.56 c	6.59±2.28 a	29.96±1.71 c	17.64±2.65 b	6.56±4.33 a
N	146.26±26.56 b	86.85±9.61 d	22.20±7.00 c	64.65±4.38 c	5.27±1.80 a	35.01±5.26 abc	14.76±8.18 b	4.98±2.07 a
P	182.56±19.90 ab	91.80±0.87 cd	20.36±0.58 c	71.45±0.29 bc	8.01±2.59 a	32.15±3.27 bc	13.88±0.59 b	6.33±2.98 a
K	179.08±40.35 ab	96.49±12.90 cd	24.09±2.28 bc	72.40±12.61 bc	7.68±1.50 a	32.09±6.38 bc	20.12±3.63 ab	7.75±0.75 a
NP	239.15±29.61 a	126.67±3.81 ab	31.44±5.45 abc	95.23±4.89 ab	6.02±1.36 a	43.33±5.42 ab	20.07±2.19 ab	12.62±9.74 a
NK	188.02±40.41 ab	97.55±17.40 cd	24.54±3.88 bc	73.01±14.73 bc	6.99±1.20 a	32.21±1.31 bc	17.42±1.70 b	9.15±4.89 a
NPK	221.90±48.88 ab	116.44±10.44 bc	26.31±9.87 abc	90.13±18.62 abc	9.80±3.96 a	42.22±9.09 ab	27.05±1.70 a	9.89±2.37 a
OM	251.94±42.81 a	138.38±8.95 ab	36.83±4.34 a	101.56±4.95 a	8.97±2.70 a	45.55±6.87 a	26.30±7.06 a	13.75±4.42 a
OM + NPK	264.67±71.17 a	145.82±26.36 a	33.80±5.49 ab	112.02±20.95 a	9.73±3.06 a	41.76±2.47 ab	21.41±1.42 ab	14.09±10.66 a

CK, without fertilization; N, mineral N fertilizer; P, mineral P fertilizer; K, mineral K fertilizer; NP, mineral NP fertilizer; NK, mineral NK fertilizer; NPK, mineral NPK fertilizer; OM, organic manure; OM + NPK, organic manure plus mineral NPK fertilizer. Values (Means ± SD, $n=3$) followed by the same letter are not significantly different within columns ($P=0.05$).

significant separation when comparing soil samples from OM, OM + NPK, NP and NPK to CK, N, P, K and NK along PC 1 (Fig. 1a). The most PLFAs were located to the right along PC1 for OM, OM + NPK, NP and NPK (Fig. 1b). The dominant PLFAs were 10Me16:0, i15:0, 16:0, 18:1 ω9, 14:0, 18:1 ω7, 17:0, cy19:0 and 18:2 ω6,9. A clear separation was also found when comparing the PLFA patterns of soil samples from NPK to OM, OM + NPK, N, P, K, NP and NK along PC 2. The dominant PLFAs 10Me19:0, i16:0 and 18:2 ω6,9 were located to the upside along PC 2 for NPK. Conversely, the dominant PLFAs a15:0 and a16:0 were negatively correlated with PC 2.

Metabolic activity and functional diversity

The AWCD was significantly increased by the application of organic manure (OM and OM + NPK) and significantly declined in the K treatment (Table 4). The functional diversity indices including Shannon index (H'), Simpson index (D) and McIntosh index (U) were also significantly increased by the application of organic manure (OM and OM + NPK) whilst significantly least in the K treatment.

An overall PCA was done for the observed substrate utilization patterns of all soil treatments from long-term field site. Two components from the PCA analysis were expressed by 39.4% and 12.1% of the overall variance (PCA axis 1 and 2) in the nine treatments (Fig. 2). Substrate utilization patterns from organic manure (OM and OM + NPK) were clearly different from organic manure deficient treatments (CK, N, P, K, NP, NK and NPK) on the PC 1 axis. Microbial communities in the K treatment were well separated from the other fertilizer treatments. Correlation analysis of the loadings of the most influential carbon sources on PC l indicated that glycogen, α-cyclodextrin, glucose-1-phosphate, β-methyl-D glucoside, D-malic acid, L-serine, L-asparagine, pyruvic acid methyl ester, L-arginine, glycyl-L-glutamic acid, putrescine and i-erythritol were positively correlated with PC l. The C sources with highest loadings on PC 2 were D,L-a-glycerol phosphate, which was also positively correlated with PC 2.

Stepwise multiple regression analysis

Stepwise multiple regression analysis showed that total N significantly affected total PLFAs, bacterial PLFAs, Gram-negative bacterial PLFAs, fungal PLFAs, Shannon

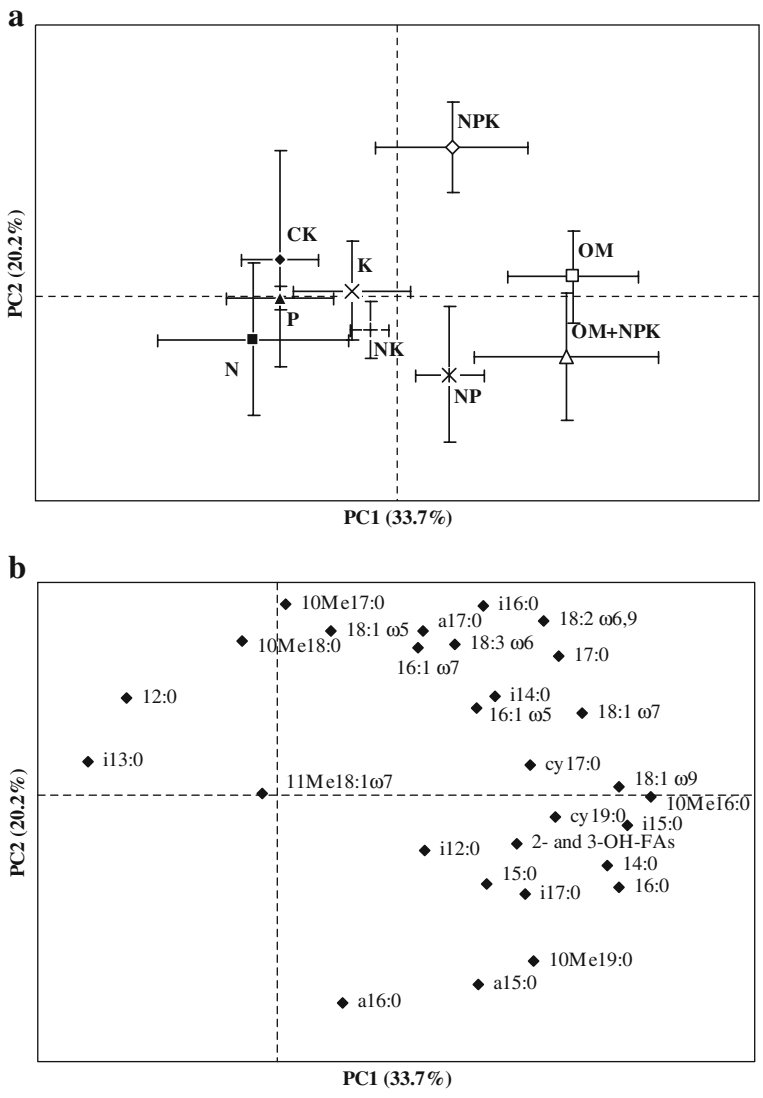

Fig. 1 **(a)** Principal component analysis (PCA) of the PLFA pattern from soils after long-term application of mineral fertilizer and organic manure. **(b)** Loadings of the individual PLFAs from the PCA of the PLFA data of principal components 1 and 2. Factor 1 accounted for 33.7%, and Factor 2 for 20.2%, of the variance. CK, without fertilization; N, mineral N fertilizer; P, mineral P fertilizer; K, mineral K fertilizer; NP, mineral NP fertilizer; NK, mineral NK fertilizer; NPK, mineral NPK fertilizer; OM, organic manure; OM + NPK, organic manure plus mineral NPK fertilizer

index, Simpson index and McIntosh index (Table 5). Bacterial PLFAs, anaerobic PLFAs and Simpson index were significantly correlated with available P, and AWCD was significantly correlated with total K. Soil pH was also an important factor which clearly influenced Gram-positive bacterial PLFAs, aerobic PLFAs, actinobacterial PLFAs, and AWCD.

Discussion

After 13 years application of inorganic fertilizers for flooded double rice crops, Zhong and Cai (2007) found that soil organic C content and crop yields could be increased to a very limited extent through chemical fertilization. They found a significant regression relationship between grain yields plus straw and soil organic C content, and thus inferred that amendment with organic materials is essential for further improving soil fertility and increasing rice crop yield as well as increasing microbial biomass and community functional diversity (Zhong and Cai 2007). Our study demonstrated that the long-term application of organic manure plus balanced fertilization with N, P and K could greatly increase the maize yields. Soil pH is widely accepted as a dominant factor that regulates soil nutrient bioavailability, vegetation community structure, and plant primary productivity (Robson 1989). Our

Table 4 Functional diversity of microbial communities in soils after long-term application of mineral fertilizer and organic manure

Treatments	AWCD	Shannon index (H')	Simpson index (D)	McIntosh index (U)
CK	0.401±0.045 b	2.832±0.045 b	0.933±0.003 a	3.215±0.309 b
N	0.404±0.103 b	2.814±0.117 b	0.930±0.010 a	3.270±0.563 b
P	0.403±0.001 b	2.672±0.801 b	0.918±0.003 a	3.568±0.068 ab
K	0.102±0.170 c	2.113±0.373 c	0.778±0.117 b	0.977±1.569 c
NP	0.423±0.214 b	2.907±0.173 ab	0.936±0.012 a	3.194±1.251 b
NK	0.379±0.014 b	2.828±0.053 b	0.930±0.002 a	3.111±0.148 b
NPK	0.429±0.124 b	2.837±0.206 b	0.932±0.014 a	3.377±0.590 b
OM	0.657±0.099 a	3.156±0.045 a	0.952±0.003 a	4.472±0.535 ab
OM + NPK	0.763±0.064 a	3.209±0.051 a	0.955±0.003 a	4.989±0.283 a

CK, without fertilization; N, mineral N fertilizer; P, mineral P fertilizer; K, mineral K fertilizer; NP, mineral NP fertilizer; NK, mineral NK fertilizer; NPK, mineral NPK fertilizer; OM, organic manure; OM + NPK, organic manure plus mineral NPK fertilizer. Values (Means ± SD, n=3) followed by the same letter are not significantly different within columns (P=0.05).

study showed that soil pH, organic C and the major soil nutrients of N, P and K were significantly increased by the application of organic manure (OM and OM + NPK) for 21 years since 1986. Soil pH, organic C and soil nutrient of N were also significantly increased by the application of mineral NPK fertilizer. By the application of organic manure for 16 years since 1989, Chu et al. (2007) found that organic C and the major soil nutrients of N, P and K were also significantly increased in a sandy loam soil. Our results validated that the amendment with organic manure was essential for improving soil organic C content and soil fertility, and in particular enhancing the crop yields.

Phospholipid fatty acids are major constituents of the membranes of all living cells, and different groups of microorganisms synthesize different varieties of PLFA through different biochemical pathways. Several studies have documented the effects of fertilization on microbial community composition using PLFA patterns (Waldrop et al. 2004; Grønli et al. 2005; Demoling et al. 2008). Significant differences were found in total PLFAs and the identified representative PLFAs among the different

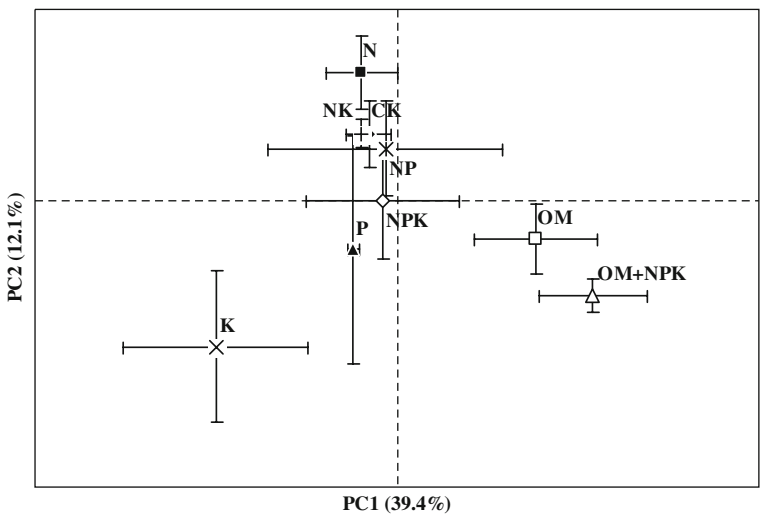

Fig. 2 Classification of treatments by PCA (n=3) in soils after long-term application of mineral fertilizer and organic manure. Factor 1 accounted for 39.4%, and Factor 2 for 12.1%, of the variance. CK, without fertilization; N, mineral N fertilizer; P, mineral P fertilizer; K, mineral K fertilizer; NP, mineral NP fertilizer; NK, mineral NK fertilizer; NPK, mineral NPK fertilizer; OM, organic manure; OM + NPK, organic manure plus mineral NPK fertilizer

Table 5 The variables which were found by stepwise regression analysis to be correlated with microbial properties or indicators in the soils from the long-term experiment

Dependents	Variables related	R^2
Total PLFA	Total N	0.402***
Bacterial PLFA	Total N, Available P	0.698***
Gram-positive bacterial PLFA	pH	0.425***
Gram-negative bacterial PLFA	Total N	0.615***
Aerobic PLFA	pH	0.244**
Anaerobic PLFA	Available P	0.415***
Fungal PLFA	Total N	0.314**
Actinobacterial PLFA	pH	0.244**
AWCD	pH, Total K	0.670***
Shannon index	Total N	0.389***
Simpson index	Total N, Available P	0.334***
McIntosh index	Total N	0.470***

, * Significant at P=0.01 and P=0.001, respectively

fertilization treatments based on the PLFA patterns. The amounts of total PLFAs, bacterial, Gram-negative and actinobacterial PLFAs were highest in the OM + NPK treatment whilst least in the N treatment, suggesting that soil prokaryote growth might be greatly stimulated by the application of organic manure plus mineral NPK fertilizer but reduced by the application of mineral N fertilizer. Long-term N fertilizer application could result in soil acidification and thus decreased the abundance and/or diversity of soil microbial communities (He et al. 2007). Soil bacteria, in particular Gram-negative bacteria that most contributed to the bacterial PLFAs, seem to be a more sensitive indicator of soil fertility than soil fungi and actinomycetes. Our finding was similar to He et al. (2008) who found that bacterial community structure and diversity were significantly influenced by long-term fertilization regimes in arable soils. The amounts of Gram-positive and anaerobic PLFAs were found to be highest in the OM treatment; however the amounts of fungal PLFAs were highest in the NPK treatment. These results may suggest that soil Gram-positive bacteria were sensitive to organic manure whilst soil fungi were sensitive to mineral fertilizer.

Gram-negative bacteria are more common than Gram-positive bacteria in the rhizosphere (Elo et al. 2000; Söderberg et al. 2004). We found an increase in the Gram-negative bacteria to Gram-positive bacteria ratio only in P-fertilized soils (P, NP, NPK, OM + NPK). A higher proportion of Gram-negative bacteria were usually interpreted as a shift from oligotrophic to more copiotrophic conditions in the soil (Borga et al. 1994; Saetre and Bååth 2000). Kourtev et al. (2003) found that the ratio of Gram-negative to Gram-positive bacteria had been related to quality of organic matter in the soil and the organic matter content of the soils decreased under blueberry, which may reflect the loss of easily decomposable materials. However, the studied soil was seriously degraded, and the fertility was very low and extremely deficient in N and P due to heavy weathering, leaching of nutrients and soil erosion. The application of mineral fertilizer to limited extent had potential to stimulate the maize growth as well as Gram-negative bacterial growth.

In our study, different fertilizer treatments also caused distinct changes in soil fungal communities. The highest contents of fungal PLFAs were observed in the NPK and least in the P treatment, indicating the importance of the balanced fertilization with N, P and K in promoting soil fungal growth. This result, however, was inconsistent with bacterial PLFAs in organic manure, suggesting that bacteria and fungi responded differently to the organic manure and mineral fertilizer. We also found that the fungi to bacteria ratio was significantly highest in the balanced fertilization with N, P and K. Overall, soil bacteria seem to be a more sensitive indicator of soil fertility than soil fungi. In contrast, actinomycetes were not significantly affected by the fertilization. Our results show that the microorganisms containing the PLFA of cy17:0, 16:0, 2- and 3-OH-FAs, 18:1 ω9, 18:2 ω6,9 and a17:0 were dominance in microbial community in all treatments. In contrast, individual PLFA 15:0, which was known to be widely distributed among bacterial taxa, was only found in the OM + NPK treatment. It suggested that the individual bacterial PLFA was sensitively responded to the organic manure plus balanced fertilization with N, P and K, and might be regarded as a sensitive indicator of soil quality.

Community level physiological profiles (CLPP) were also used increasingly to characterize microbial communities (Garland 1997; Zhong and Cai 2007; Shen et al. 2008). In agreement with PLFA profiles, our results demonstrated that fertilization also had great effects on the CLPP of the bacterial communities in soils. Long-term fertilization of organic manure (OM, OM + NPK) led to C utilization pattern shifts and increased soil microbial functional diversity. These results suggested that soil bacteria were one of

sensitive indicators of soil fertility. He et al. (2008) found that soil fungi were a more sensitive indicator of soil fertility than soil bacteria in an upland red soil based on culture-dependent and culture-independent approaches. The land use type, however, was peanut with fallow in winter. In our study, the land utilization of double maize may generate more root exudates than that of peanut. Root exudates released into soil have important functions in promoting the growth of soil microorganisms. Furthermore, maize biomass was larger and thus demands of three main nutrients, in particular N, were much more than that of peanut. The long-term application of organic manure significantly increased soil nutrient of N and met the demand of maize growth, and thus promoted soil microbial biomass, activity and diversity through abundant root exudates. Soil nutrient of P could be considered as a key factor to control the microbial biomass and diversity in subtropical red soils (Zhong and Cai 2007; He et al. 2008). Stepwise regression analysis showed that soil nutrient of N was also another key factor to control the microbial biomass and diversity in the upland red soil, which was seriously degraded and the fertility was very low and extremely deficient in N and P. However, the soil microbial biomass, activity and diversity as well as soil fertility were increased to a very limited extent by the direct application of the mineral N, P and K fertilizer. The application of organic manure may improve soil structure and function, enhance water and nutrient supplying capacity, and thus promote plant growth and maintains high crop yields. Consequently, greater microbial population and diversity were sustained by the abundant root exudates released into the soil by the maize plants. This research will also provide valuable data on the effects of mineral fertilizer and organic manure on soil bacterial diversity and relationships between diversity and crop yields. This may improve the yields and land use sustainability by allowing farmers to better match mineral fertilizer and organic manure with crop demand in degraded red soils.

In conclusion, long-term fertilization had great effects on soil microbial biomass, activity and diversity. The mineral fertilizers and organic manure could affect the microbial parameters in different ways. The long-term application of organic manure leads to soil microbial community shifts and increased soil microbial biomass, activity and diversity. Most microbial parameters were mainly correlated with total N and available P, which were seriously deficient in the red soil derived from quaternary red clay, indicating that the application of organic manure plus mineral NPK fertilizer could affect microbial parameters indirectly by increasing the contents of these critical nutrients. Our results could provide a better understanding of the importance of organic manure plus balanced fertilization with N, P and K in promoting the soil microbial biomass, activity and diversity and thus enhancing crop growth and production.

Acknowledgements This work was supported by a Knowledge Innovation Project of the Chinese Academy of Sciences (No. Kzcx2-yw-408) and the National Natural Science Foundation of China (No. 40830531). The authors thank two anonymous reviewers for their valuable comments and suggestions that had improved this manuscript greatly.

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