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Phospholipid fatty acid patterns of microbial communities in paddy soil under different fertilizer treatments

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Abstract

The microbial communities under irrigated rice cropping with different fertilizer treatments, including control (CK), PK, NK, NP, NPK fertilization, were investigated using phospholipid fatty acid (PLFA) profile method. The results of this study revealed that the fertilizer practice had an impact on the community structure of specific microbial groups. The principal components analysis (PCA) showed that proportion of the actinomycete PLFAs (10Me 18:0 and 10Me 16:0) were the lowest in the PK treatment and the highest in the NPK treatment, which means that soil nitrogen status affected the diversity of actinomycetes, whereas nitrogen cycling was related to the actinomycets. Under CK treatment, the ratio of Gram-positive to Gram-negative bacteria was lower compared with that in fertilizer addition treatments, indicating that fertilizer application stimulated Gram-positive bacterial population in paddy soil. The fatty acid 18:2ω6,9, which is considered to be predominantly of fungal origin, was at low level in all the treatments. The ratio of cy19:0 to 18:1ω7, which has been proposed as an indicator of stress conditions, decreased in PK treatment. Changes of soil microbial community under different fertilizer treatments of paddy soil were detected in this study; however, the causes that lead to changes in the microbial community still needs further study.

Key words: paddy soil; fertilizers; soil microorganisms; community structure; PLFA; PCA

Introduction

Microorganisms are important living components of the soil, as they play a key role in cycling of plant nutrients. Soil microbial biomass is considered to act both as the agent of biochemical changes in soil and as a repository of plant nutrients such as nitrogen (N) and phosphorus (P) in agricultural ecosystems (Zhou *et al*., 2002; Chen *et al*., 2004). Numerous studies gave an account of the relationship between soil fertility and microbial biomass (Brookes, 1995). Recent methodological advances such as analysis of DNA and PLFAs as well as cultivation on Biolog Gram-negative (GN)-plates allow more detailed information on soil microbial activities and community structure (Widmer *et al*., 2001; Zhang *et al*., 2005). However, most studies on microbial community structure using these analysis methods were focused on polluted soil (Frostedård *et al*., 1993) and soil management practices (Mendum *et al*., 1999; Bossio, 1998), and there were quite few studies on nutrient cycling and ecology of irrigated rice system. The composition of the microbial community, in particular the proportion of bacteria and fungi, may influence C and N turnover of paddy soil. Recently, Erica *et al*. (2005) have found that actinomycetes have been significantly correlated with gross NH_4^+ mineralization in forest soil. However, the precise mechanisms driving N-fertilizer-mediated microbial community changes still remain unclear.

In the present study, paddy soil was treated with different combinations of NPK fertilizers, including CK, PK, NP, NK, and NPK in a long-term field experiment. The PLFA patterns were determined after six years of consecutive cropping to assess the characteristics of soil microbial ecosystem.

1 Materials and methods

1.1 Experimental design

A long-term field experiment was launched in 1998 in the suburb of Jinhua City of Zhejiang Province, China. The soil of the site belongs to Typic Eduoagulpt of alluvial origin. Before the experiment began, composite soil sample was collected from 0–15 cm depth to determine basic physical-chemical properties (Table 1). The experiment comprised five fertilizer treatments (Control, PK, NP, NK, and NPK), arranged in a randomized complete block design (RCBD) with three replications. Each treatment plot area was 45 m^2 and rice hill spacing was 20 cm×20 cm. Urea was used as N fertilizer (150 kg N/hm^2 for early rice and 180 kg N/hm^2 for late rice); 50% of N was applied one day before transplantion as basal dose, 25%

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Table 1 Physical and chemical characteristics of the basic soil

Soil type	PH 1:1 H_2O	Total N (g/kg)	Available-N (mg/kg)	Olsen's-P (mg/kg)	Available-K (mg/kg)	Sand (g/kg)	Silt (g/kg)	Clay (g/kg)
Paddy soil	4.8	2.7	123.4	16.5	54.6	278	562	160

at pre-tillering and 25% at panicle initiation stage. Super phosphate (25 kg P/hm^2) was applied as basal fertilizer; and KCl (100 kg K/hm^2) was used as fertilizer K, 50% of which was applied as basal fertilizer and 50% at panicle initiation stage. Soil samples (0–15 cm) of all the plots were collected after harvest of late rice in 2004 and were analyzed.

1.2 Phospholipid fatty acid (PLFA) analysis

Lipid extraction and PLFA analysis were performed using a modification of Bligh and Dyer (1959) method as described by Bossio *et al*. (1998). In brief, 2.0 g (dry equivalent weight) was extracted with a chloroform-methanol-citrate butter mixture (1:2:0.8), and the phospholipids were separated from other lipids on a silicic acid column. Phospholipid phosphate was determined using the spectrometric method. Whereas, the phospholipids were subjected to a mild-alkali methanolysis, and the resulting fatty acid methyl esters were separated by gas chromatography (Yao, 2000a).

Fatty acids are designated in terms of total number of carbon atoms with the number of double bonds given after a colon. The position of the double bond is defined by the symbol ω followed by the number of carbons from the methyl end of the fatty acid molecule. *Cis*- and *trans* configurations are indicated by c and t; the i and a refer to *iso*- and *anteiso* branching; br indicates an unknown branch position; and cy refers to cyclopropyl fatty acids. Hydroxy groups are indicated by 'OH'. 10Me indicates a methyl group on the 10th carbon atom from the carboxyl end of the molecule (Arao, 1999; Bååth and Anderson, 2003; Steenwerth *et al*., 2003).

1.3 Statistics

PLFA data were analyzed using principal components analysis (PCA) to elucidate major variation and covariation for individual PLFA employing varimax rotation. This was performed on $\log_{10}$ transformed mole percentages of individual PLFAs. PCA scores were subsequently analyzed by analysis of variance (ANOVA) with correlation matrix using SPSS Version 11.5. Cluster analysis was performed by SPSS software.

2 Results

The soil under each treatment contained various PLFAs composed of saturated, unsaturated, methyl-branched and cyclopropane fatty acids (Fig.1). Twenty-nine PLFAs with chain lengths ranging from C_{12} to C_{20} were identified. The PLFAs patterns varied in response to different fertilizer treatments, as revealed by their relative abundance. For example, the proportion, expressed in mol%, of the branched i14:0 and i15:0 was higher in the NPK treatment than that in other treatments, whereas the proportion of the monounsaturated 16:1ω7 and 16:1ω5 was higher in the PK treatment (Fig.1).

The ratios of Gram-positive to Gram-negative bacteria (the sum of mole percent of i15:0, 10Me 16:0, i17:0, a17:0, 10Me 17:0, a15:0, i16:0, i14:0 to that for 16:1ω9, 16:1ω5, cy17:0, 18:1ω7, 16:1ω7, cy19:0ω8) were calculated as described by Frostegård *et al*. (1996). The ratio of Gram-positive to Gram-negative bacteria was significantly higher under the NPK treatment compared with other treatments (Fig.2), and no significant difference between NP, NK, and PK treatments could be detected (at the 0.05 level). The control (with no fertilizer) had the smallest proportion of Gram-positive to Gram-negative bacteria, suggesting that fertilizer application stimulated Gram-positive bacterial population in paddy soil. The PLFAs cy19:0 and 18:1ω7c are signature fatty acids of Gram-negative bacteria; 18:1ω7c is the precursor molecule of cy19:0. The ratio of cy19:0 to 18:1ω7c was significantly higher under NPK than that under PK treatment (Fig.3).

The PLFA concentration data (as l g mol percentages) were subjected to principal component analysis (PCA). Most of the variation in PLFA patterns was due to different fertilizer application and could be explained by the first two principal components. The ordination plot in Fig.4 illustrates the difference in the PLFA composition of the five treatments, where PC1 and PC2 account for 39.27% and 24.12% of the variation, respectively. The N fertilizer treatments (NP, NK, and NPK) were found on the right-hand side of the plot, and the NPK treatment was in the first quadrant, whereas the PK treatment was found on the left-hand side of the plot. The points representing NK and NP were closer, although NK was in the first quadrant. The NP was in the fourth quadrant. The ordination plot showed that NP, NK, and CK treatments data formed a cluster, and also NPK treatment formed a cluster and had positive scores for PC1 and PC2, whereas data points for PK clusters were away from the other four treatments and had negative scores for PC1. It was fitting to separate five fertilizer treatments into three kinds by cluster analysis. It suggested that microbial community composition might be dissimilar under different fertilizer treatments except under NP and NK treatments.

Principal components analysis also identified fatty acids that were important in explaining the variability in PLFA profiles (Fig.5). Certain specific PLFAs that were identified, including 15:0, 17:0, 20:0, the branched i17:0, the methyl-branched 16:0 (10Me), 18:0 (10Me), and monounsaturated 17:1ω8c, as well as cyclopropane cy17:0, cy19:0 were found on the right-hand side in the plot. Except 18:0 (10Me), all these PLFAs are common in bacteria, indicating that the bacterial population increased with N treatments, especially under NPK treatment. Fatty

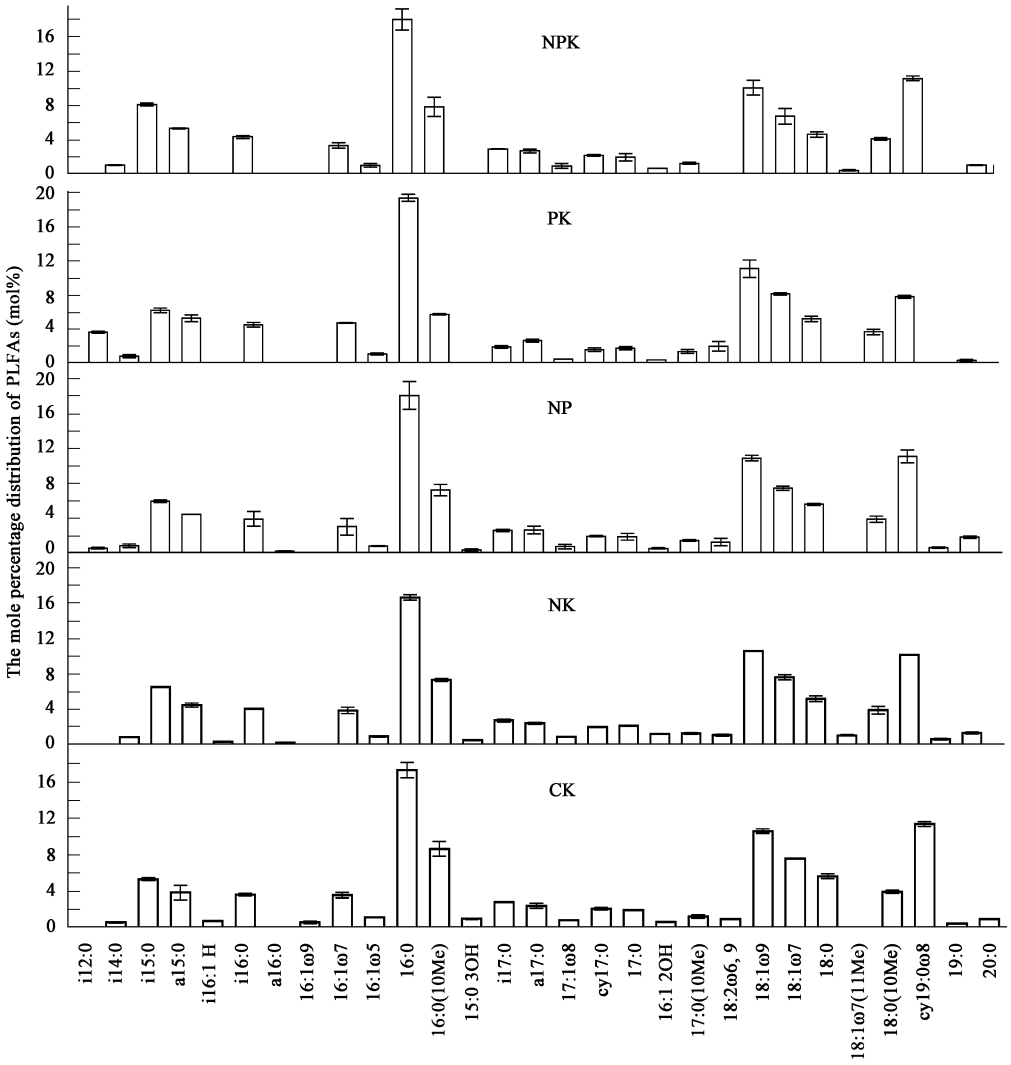

Fig. 1 Mol% of different PLFAs under five fertilizer treatments.

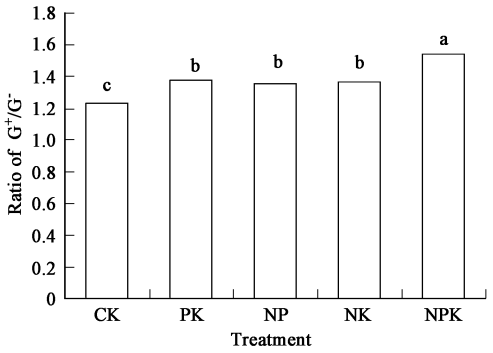

Fig. 2 Ratio of Gram-postive to Gram-negative PLFAs under five fertilizer treatments. The same letter means there are no significant differences at 0.05 level.

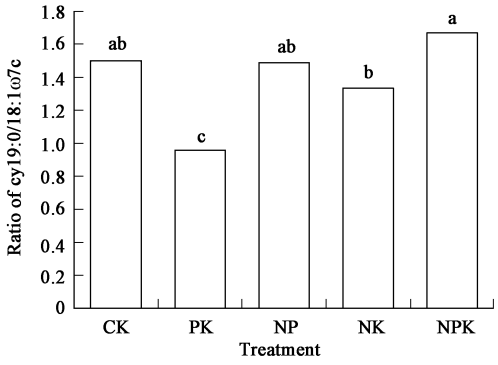

Fig. 3 Ratio of cy19:0 to 18:1ω7c under five fertilizer treatments. The letters a, b, and c are the same as Fig.2.

acids with a 10Me branching are particularly abundant in actinomycetes (Kroppenstedt, 1985; Francisco *et al*, 2000). 10Me16:0 may also occur in sulfate-reducing bacteria (Findlay and Dobbs, 1993). The average mole percentage of actinomycetes PLFA 10Me18:0 under balanced fertilization (NPK) was the highest, which was 11.4% higher than that under PK treatment.

The PLFAs 16:0, the branched i16:0, a15:0, and the monounsaturated 16:1ω7, 18:2ω6,9, 18:1ω9, 18:1ω7, were found on the left-hand side of the plot (Fig.5). Thus, these PLFAs were more abundant under PK treatment. The PLFAs i16:0 and a15:0 are common in Gram-positive bacteria, whereas unsaturated fatty acids are common in Gram-negative bacteria. The PLFAs 16:0 has not been associated with any particular groups of microorganisms

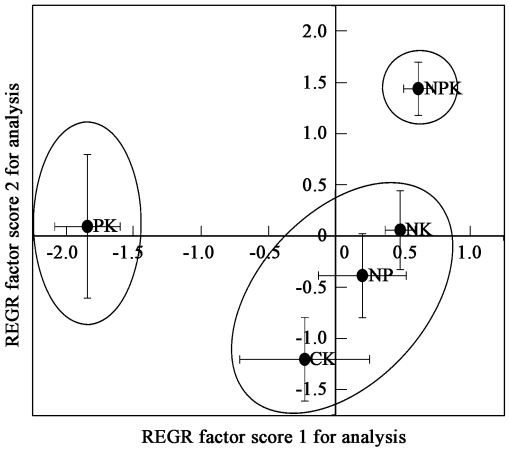

Fig. 4 PCA showing variations in PLFA pattern under different fertilizer treatments.

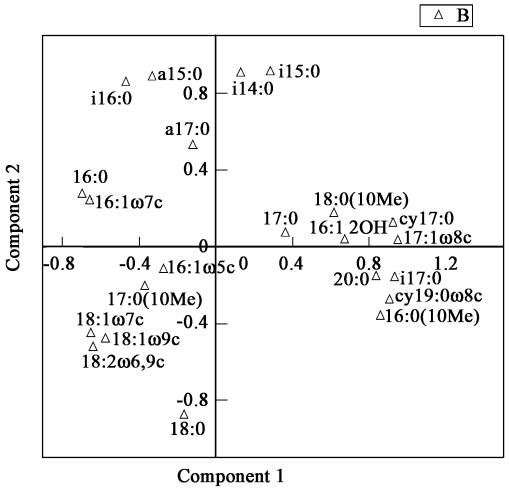

Fig. 5 PCA showing loading values for individual PLFAs.

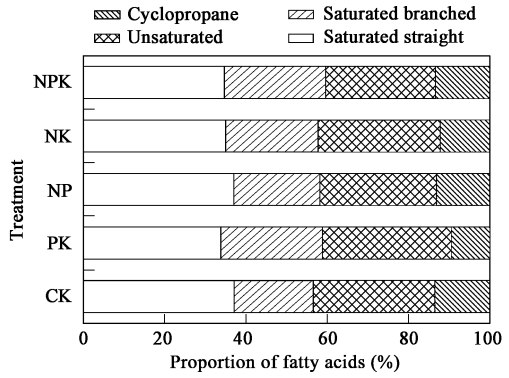

Fig. 6 Proportion of fatty acids divided into saturated, branched, unsaturated and cyclopropane acids.

(Feng *et al*., 2003). The fatty acid 18:2ω6,9 and 18:1ω9 are the fungal molecular markers used in this study (Frostegård and Baath, 1996). The PC loadings showed that fungi were strongly negatively correlated (loading –0.64 and –0.50, respectively) with PC1 (the first principal component), indicating that these fungi tended to decrease under the NPK treatment and increase under the PK treatment. Along PC2 (the second principle component) axis, the branched i14:0, i15:0, a15:0, i16:0, and a17:0 were strongly positively correlated (i.e., loading 0.7 or greater) with PC2, whereas 18:0, 18:1ω7, 19:1ω9 were negatively correlated (i.e., loading –0.5 or less) with PC2. No PLFA were found very close to, or on the origin in the PCA plots, indicating that all PLFAs identified were affected by different fertilizer treatments. The proportion of unsaturated fatty acids was the highest (31.7%) under PK, whereas lowest (26.9%) under NPK treatment (Fig.6). In contrast, the proportion of cyclopropane fatty acids was relatively lower under PK treatment.

3 Discussion

The PLFA and Biolog analyses are commonly used analytical procedures for the evaluation of soil biological characteristics (Frostegård and Bååth, 1996; Yao *et al*., 2000a; Zak, 2000; Christopher *et al*., 2003). PLFA analysis gives more information than methods such as plate counts, and is regarded as the best method for assessing a broad-spectrum of difference in community (Yao *et al*., 2000a, b). Using Biolog method, Zhang and Wang (2005) observed that soil nutrient deficiency and unbalanced fertilization had negative effects on the diversity of the microbial community. In the current study, the microbial community was further assessed using PLFA. The main finding of this study is that different fertilizer practices have an impact on the community structure of specific microbial groups. The actinomycetes PLFA 10Me 18:0 and 10Me 16:0 showed the lowest abundance under the PK treatment, whereas relatively greater abundance under NPK treatment. At this study site, the PK plot showed severe N deficiency after five years of consecutive rice cropping (Zhang and Wang, 2005). This study suggested that PLFAs (both 10Me18:0 and 10Me16:0) were abundant under N treatments, especially the NPK treatment. Recently, Erica *et al*. (2005) found that lipid biomarkers that most likely represent the actinomycetes guild (both 10Me18:0 and 10Me16:0) were significantly correlated with gross NH_4^+ mineralization. This study suggested that soil indigenous N supply and N fertilization practice had an impact on the diversity of actinomycetes in soils. However, Cristopher *et al*. (2003) reported that the actinomycetes PLFA 10Me18:0 was more abundant in the unfertilized grassland soils. Previous studies, however, suggested that the abundance of 10Me18:0 changed under liming (Frostegafird *et al*., 1993) and with soil depth (Fritze *et al*., 2000). The results of this study suggest that the status of soil N can also change the structure of microbial communities in paddy soils.

The amounts of 18:2ω6,9 in this study ranged from 0.95 to 2.42 mol% and were lower than those reported (Frostegård and Bååth, 1996; Yao *et al*., 2000a), which were>2.5 mol%. This indicated that paddy soil might have low fungal biomass, although it varied under different fertilizer treatments. 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 stationary growth, acidic condition, low oxygen, and high temperature (Guckert *et al*., 1986). A significant decrease

in this ratio occurred under PK treatment compared with other fertilizer treatments; however, the cause leading to this decrease is not known and needs further research.

Branched fatty acids were used as indicators of Gram-positive bacterial biomass, and the amounts of fatty acids 18:1ω7, 16:1ω7, cy17:0, and cy19:0 were used as indicators of Gram-negative bacteria (Frostegård and Bååth, 1996). In this study, the principal component analysis (Figs.4 and 5) indicated that the NPK treatment increased the percentage of cyclopropane fatty acids (cy17:0, cy19:0), whereas it decreased the percentage of unsaturated fatty acids.

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