Responses of PLFA and NLFA to Fertilization in a Chinese Arable Mollisol

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Abstract
The effects of 17y pig manure (PM) (organic matter, 9 t/hm^2 each year) and inorganic fertilizer [N, 150 kg/hm^2 each year (urea); P, 32.73 kg/hm^2 each year (calcium hydrogen phosphate)] amendments on phospholipids fatty acid (PLFA) and neutral lipid fatty acid (NLFA) in different soil microbial communities were investigated. The fertilizer treatments were as follows: CK, N (nitrogen), NP (nitrogen + phosphorus), MCK (PM only), MN (PM + nitrogen), MNP (PM + nitrogen + phosphorus). The result of PLFA analysis showed that the addition of PM, nitrogen or phosphorus fertilizer significantly increased soil microbial biomass. As fungal-to-bacterial PLFA ratio for CK was dramatically higher than for the PM treatments, it was reasonable that the fungi might be adapted to nutritional deficiency more easily than bacteria. As 17 years of cropping with single N application resulted in low P availability and led to a great increase in fungal NLFA contents, P was confirmed to be the important limiting nutrient for fungal growth in this arable Mollisol.

Key Words
PLFA, NLFA, soil microorganisms, fertilization, Chinese Mollisol.

Introduction
As one of three major black soils in the world, Chinese Mollisol's cultivated area is 7×10^6 hm^2 which accounts for above 50% of total cultivated areas of Heilongjiang and Jilin province of China. Soil microorganisms in this Mollisol under different fertilization and land management practices have been studied mainly about soil microbial biomass, soil enzymes activities and quantity changes of culturable microbes (Li *et al.* 2004; Shi *et al.* 2004). Fewer articles about the living soil microbial community structure and physiological status affected by different fertilization were reported in this area. The objectives of this study are to find out: 1. How the native fungi and bacteria react to the pig manure (PM) and chemical fertilizers application, 2. Weather the NLFA or NLFA/PLFA ratio can be used to indicate the physiological status of not only soil fungi but also soil bacteria, and 3. Which kind of soil nutrients is absolutely necessary to microbial community?

Methods
Basic properties
Total carbon and nitrogen contents were determined by dry combustion using a C/N analyzer, the pH was measured in a 1:1 soil/water suspension, readily available K was extracted by ammonium acetate and determined by flame photometer method, available P was extracted by sodium bicarbonate and determined by molybdate blue colorimeter method, available N was indicated as alkali-hydrolysable nitrogen (Lu RK 1999). Soil microbial biomass C (SMB-C) or soil microbial biomass N (SMB-N) was determined by the chloroform fumigation-extraction method using a 0.5 M K_2SO_4 extracting solution (Brookes *et al.* 1985). SMB-C and SMB-N were calculated using extraction efficiency factor (K_{ec}) of 0.38 and 0.45 respectively. The soil physical and chemical properties were shown in Table 1.

PLFA and NLFA analysis
NLFA and PLFA analyses were performed using the modified Bligh and Dyer method (Bligh and Dyer, 1959; Frostegard *et al.* 1993).

Table 1. Basic soil properties of 6 fertilization treatments.

Treatment	pH	Organic matter	Total N	Alkalytic N	Available P	SMB-N	SMB-C
		g/kg		mg/kg			
CK	8.43	32.86	1.656	95.92	2.009	12.90	92.47
N	8.40	33.84	1.757	112.5	1.467	13.62	107.8
NP	8.41	33.01	1.747	99.15	4.207	16.21	113.0
MCK	8.08	49.63	2.479	175.3	100.2	27.92	203.2
MN	7.68	54.87	2.633	182.2	114.2	44.55	235.6
MNP	7.68	56.93	2.954	210.0	109.6	37.68	274.5

Note: 1. All values about soils are given as dry weight. 2. SMB-C, soil microbial biomass carbon; SMB-N, soil microbial biomass nitrogen.

Results

3.1 PLFA contents in different fertilizer amendments

PLFA 16:0 (BioPLFA), a biomarker of soil viable microbial biomass, increased significantly with PM amendment as the amounts of PLFAs in MCK (4.53 nmol/g) and MN (5.05 nmol/g) and MNP (4.62 nmol/g) were much higher than those of CK (2.6 nmol/g) and N (3.06 nmol/g) and NP (3.18 nmol/g). In spite of the order in no-PM system (NP>N>CK), the increase of PLFA 16:0 in MN was greater than MNP (MN > MNP > MCK) (Figure 1). The trend of total bacterial PLFAs (bacPLFA) of i15:0, a15:0, 15:0, i16:0, 16:1ω9, i17:0, cy17:0 in different fertilizer amendments were very similar to that of PLFA 16:0, as MN (10.3 nmol/g) and MNP (9.83 nmol/g) were significantly higher than MCK (8.85 nmol/g), and NP (7.62 nmol/g) > N(5.97 nmol/g) > CK (4.95 nmol/g) (Figure 1). The fungal PLFAs (FunPLFA), indicated as the sum of 18:2ω6,9 and 18:1ω9, were also significantly promoted by PM addition. The highest ones occurred in MNP (6.26 nmol/g), and the amounts of MN (5.7 nmol/g) and MCK (5.48 nmol/g) were significantly higher than those of N (3.8 nmol/g), NP (3.77 nmol/g) and CK (3.59 nmol/g) (Figure 1). In addition, the application of chemical fertilizers did not significantly affect fungal PLFAs contents in no-PM system. The fungal-to-bacterial PLFA ratios (Fun/bac), a measure of the sum of 18:2ω6,9 and 18:1ω9 to total bacterial PLFAs of i15:0, a15:0, 15:0, i16:0, 16:1ω9, i17:0, cy17:0, did not show great variation among PM or no-PM system, except that NP (0.49) was significantly lower than CK (0.73) (Figure 1).

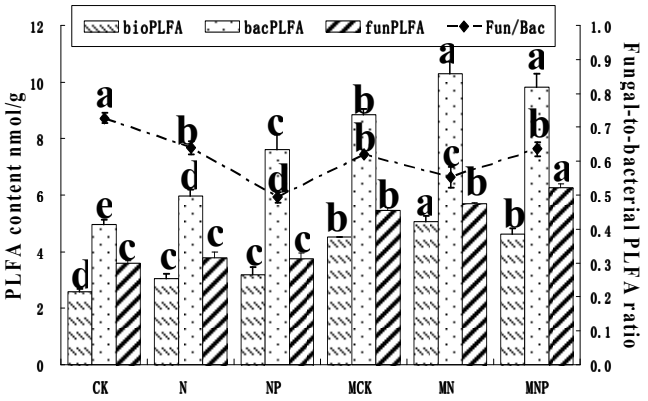

Figure 1. PLFAs of biomass, fungi and bacteria in different treatments.

3.2 NLFA contents in different fertilizer amendments

Microbial biomass biomarker NLFA 16:0 decreased with PM amendment [N (5.2 nmol/g) > MN (4.42 nmol/g) or CK (4.58 nmol/g) > MCK (4.19 nmol/g)] except those treated with P [MNP (5.14 nmol/g) > NP (2.54 nmol/g)] (Figure 2). Although combination of PM and NP increase NLFA 16:0 significantly (MNP>CK), mineral N+P dramatically decreased NLFA 16:0 (CK>NP). The sum of NLFAs i15:0, a15:0, 15:0, i16:0, 16:1ω9, i17:0, cy17:0, indicating total bacterial NLFAs, were much higher in PM amendments than no-PM, as the order was MNP (14.02 nmol/g) > NP (4.44 nmol/g , MN (10.40 nmol/g) > N (7.05 nmol/g), MCK (9.75 nmol/g) > CK(5.29 nmol/g) (Figure 2). Chemical N amendment stimulated bacterial NLFA but N+P inhibited the bacterial NLFAs (N>CK>NP). The fungal NLFAs of 18:2ω6,9 and 18:1ω9, decreased significantly with PM addition [N (8.73 nmol/g) > MN (4.49 nmol/g) or CK (6.13 nmol/g) > MCK (4.91 nmol/g)] except the amendments with NP [MNP (6.94 nmol/g) > NP (4.70 nmol/g)](Figure 2). The amount of fungal NLFAs in chemical N amendment was nearly 1 times higher than NP or MN. The fungal-to-bacterial NLFA ratios, a measure of the sum of 18:2ω6,9 and 18:1ω9 to total bacterial NLFAs of i15:0,

a15:0, 15:0, i16:0, 16:1ω9, i17:0, cy17:0, decreased dramatically almost 1 to 2 times in PM amendments, as the order was N (1.24) > MN (0.43), CK (1.16) > MCK (0.50), NP (1.06) >MNP (0.50) (Figure 2). The application of single N fertilizer increased the fungal-to-bacterial NLFA ratio, but NP decrease it (N>CK>NP), and there was no great variations among the ratios of PM system.

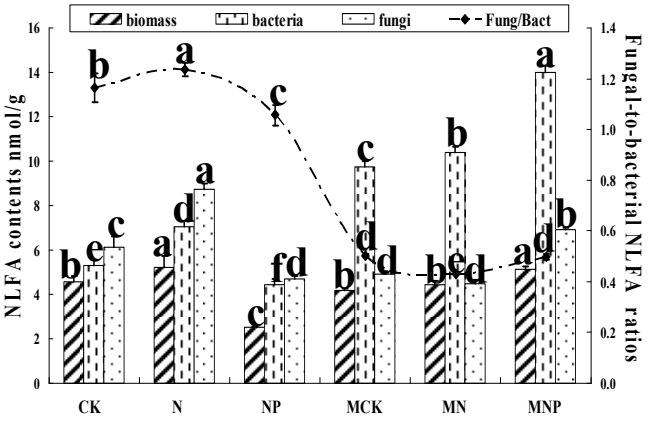

Figure 2. NLFAs of biomass, fungi and bacteria in different treatments

3.3 PCA of PLFA (nmol%) and NLFA/PLFA ratios

Principal components analysis (PCA) of 13 different PLFAs did not differentiate clearly PM from no-PM treatments (Figure 3a), while PCA of NLFAs and NLFA/PLFA ratios did divide no-PM and PM treatments into two parts: the first principal component had a significant PM effect, as the addition of the PM shifted the treatments to the right; while the second component was affected significantly by inorganic fertilizer, as CK, N and NP treatments were found to the upper PC graph (Figure 3c, 3e).

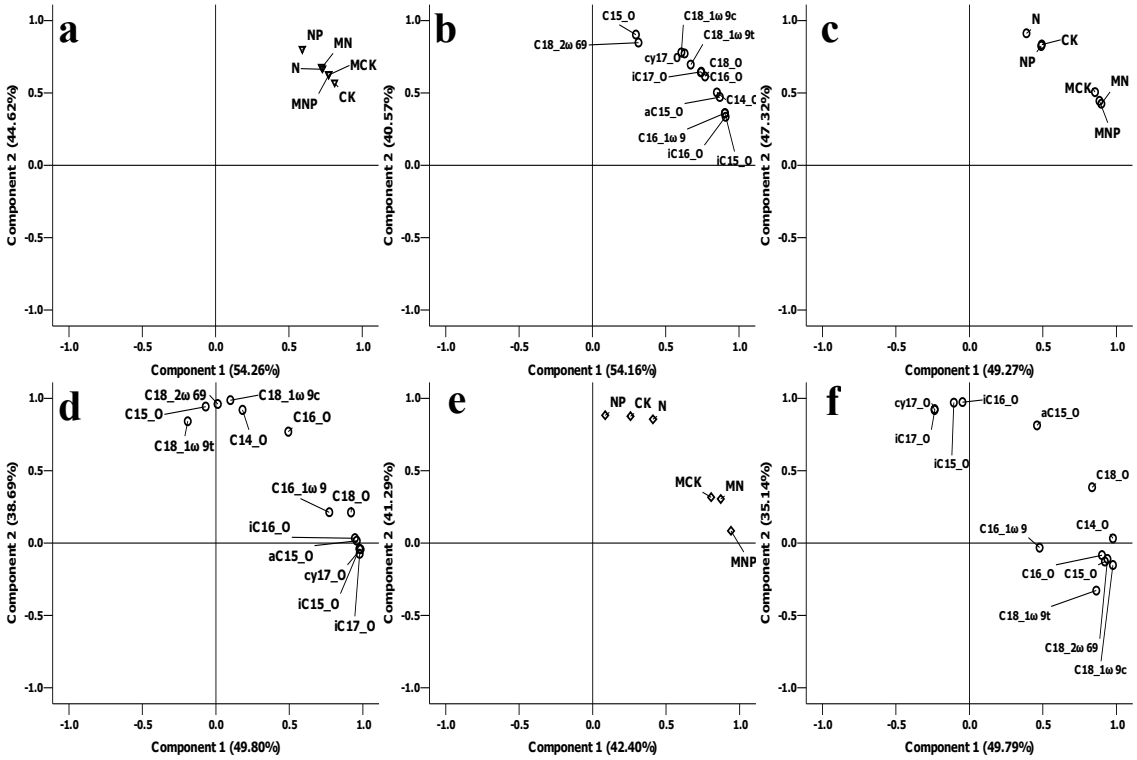

Figure 3. Principal component analysis of fatty acids showing score plots for the different treatments and loading values for the individual fatty acids

The loading values for the individual PLFAs, NLFAs and NLFA/PLFA ratios changed in different ways (Figure 3b, 3d, 3f). As for PLFAs, i16:0, i15:0 and 16:1ω9 increased with CK, while 15:0 and 18:2ω6,9 increased greatly with NP treatment. However, i15:0, a15:0, i16:0, i17:0, cy17:0 and 18:0 NLFAs significantly increased with PM application, and 14:0, 15:0, 18:2ω6,9, 18:1ω9c and 18:1ω9t NLFAs increased with chemical fertilizers application. Loading values of the NLFA/PLFA ratios PCA indicated that 14:0, 15:0, 16:0, 18:2ω6,9, 18:1ω9c, 18:1ω9t and 18:0 were mainly influenced by PM addition, while i15:0, i16:0, i17:0, cy17:0 and a15:0 were significantly affected by chemical fertilizers application.

Conclusion

The live soil microorganisms were dramatically affected by 17y PM and chemical fertilizer amendments with the great variation of soil basic properties such as pH, organic matter, total N and available P. Available P was the most important limiting factor for the variation of soil microbial physiological condition. Though fungi were much more tolerant to the nutrient deficiency than bacteria, PM plus chemical nutrients dramatically activated fungal growth. The NLFA contents are particularly useful to indicate the Mollisol bacterial and fungal physiological status. The pathway of fungal or bacterial NLFAs accumulation in the condition of nutritional deficiency was very different from that in the nutritional sufficiency. PCA of NLFA-to-PLFA ratios was more useful than NLFAs or PLFAs to determine the change of microbial community structure and physiological status caused by the long-term application of different fertilizers.

It was obvious that the long term application of PM with or without chemical fertilizers had changed the pathway of organic matter and energy transformation in the different communities of soil microbes by changing the microbial structures.

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