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Effect of different fertilization treatments on ecological characteristics of microorganism in paddy soil

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Abstract: Investigation of the characteristics of soil microorganism ecosystem under irrigated rice cropping with different fertilization treatments showed that balanced application of N, P and K promoted microbial biomass and community composition, while unbalanced fertilization reduced microbial N and increased C/N ratio of the microbial biomass and that the fertilizer practice had impact on the community structure of specific microbial groups and the microbe diversity in soils. This research focused on soil microbial biomass and soil microbial community structure in a long-term fertilization experiment on rice based on nutrient balance concepts.

Key words: Fertilization, Paddy soil, Soil microorganism
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INTRODUCTION

Soil microorganism is an important soil component because it plays a key role in soil nutrient cycling. Soil microbial biomass is considered to serve both as agent of biochemical changes in soil and as a repository of plant nutrients such as nitrogen (N) and phosphorus (P) in agricultural ecosystems (Jenkinson and Ladd, 1981). Microbial population in the soil-floodwater-rice system perhaps comprises one of the most complex biological systems in agriculture. A few studies have revealed that microbial biomass is an important source of mineralizable soil organic N in paddy soils (Marumoto, 1984), and studies also have reported on the relationship between soil fertility and microbial biomass (Brookes *et al*., 1985). More recently, methodological advances such as phospholipid fatty acid (PLFA) and Biolog gram-negative (GN)-plates allow for more detailed information on soil microbial activities involved in community structure (Widmer *et al*., 2001). However, most studies on microbial community structure using these analysis methods have focused on polluted soil (Frostedård *et al*., 1993) and on soil management practices (Mendum *et al*., 1999; Bossio *et al*., 1998), there have been few studies on nutrient cycling and ecological characteristics of microorganism in irrigated rice system.

This research focused on soil microbial biomass and soil microbial community structure in a long-term fertilization experiment on rice based on nutrient balance concepts.

MATERIALS AND METHODS

Experimental design

A long-term field NPK experiment was established in 1998 in the suburb of Jinhua City of Zhejiang Province. The soil was a Typic Eduoagulpt of alluvial origin. Initial soil properties in spring of 1998 (0~15 cm depth) were 278 g/kg sand, 562 g/kg silt, 160 g/kg clay, pH (1:1 water) 4.8, 2.7 g/kg total N, 123.4 mg/kg available-N, 16.5 mg/kg Olsen-P and 54.6 mg/kg available-K. The experiment in-

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volved five fertilizer treatments (control, PK, NP, NK, and NPK), arranged as a randomized block with three replicates. The plot area was 45 m^2 and rice hill spacing was 20 cm×20 cm. Urea was used as fertilizer N (150 kg N/ha for early rice and 180 kg N/ha for late rice), with 50% of it applied as basal fertilizer one day before transplanting, 25% at early-tillering and 25% at panicle initiation stage. Superphosphate (25 kg P/ha) was applied as basal fertilizer. KCl (100 kg K/ha) was used as fertilizer K with 50% of it applied as basal fertilizer and 50% at panicle initiation stage. Soil samples (0~15 cm) of all plots were collected after harvest of late rice in 2004 and soil microbial biomass and microbial community were analyzed.

Soil microbial biomass determination

Soil microbial biomass was measured using the fumigation extraction method. Fifteen grams moist soil were extracted immediately after sampling by shaking for 30 min with 60 ml of 0.5 mol/L K_2SO_4 and 15 g were fumigated for 24 h with ethanol-free chloroform and then extracted as described above. Details of the process can be found in (Brookes *et al*., 1985).

Biolog analysis

The BIOLOG GN micro-plates used in this study rely on redox dye tetrazolium violet to detect respiration of sole carbon sources. The 96-well GN micro-plate consists of 95 substrate-containing wells and a control well without a carbon source. Fifteen grams of soil samples were diluted to 10^{-4} solution, and inoculated into plates, which were then kept at room temperature (25 °C). Color formation in micro-plate wells (absorbance at 405 nm) was analyzed using a Biotek EL 320 micro-plate reader with an automated stracker-loader cassette. Overall color development in BIOLOG plates was expressed as average well color development (AWCD). We used two forms of ordination—principal component analysis (PCA) and canonical variate analysis (CVA)—to visualize the relationships among samples by Genstat 5.3 (NAG Ltd., Oxford, UK) (Yao *et al*., 2000).

Phospholipid fatty acid (PLFA) analysis

Duplicate 2 g field-moist soil samples were extracted with a one-phase solvent extractant, using a modification of the Bligh and Dyer (1959) method as described in (Bossio *et al*., 1998).

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; Steenwerth *et al*., 2003).

RESULTS AND DISCUSSION

Soil microbial C, N, and C/N ratio

The levels of soil microbial biomass carbon, nitrogen and C/N ratio in the different treatments are shown in Fig.1. The content of microbial carbon and also nitrogen in different treatments were significantly different by DMRT at 5% level. Soil microbial C was maximum in the NP treatments, with CK and PK treatments being second. The soil microbial biomass N showed a marked increase in the NPK treatment as compared to values in the unbalanced nutrient treatments (NP, PK and NK). The C to N ratio in NK (8.7) and NPK (7.6) treatments were markedly lower than other treatments. With continuous rice cropping, the NP plot revealed potassium deficiency, and the PK plot revealed nitrogen deficiency at the very beginning of the trial, while the NK and NPK plot did not show nutrient deficiency (Zhang and Wang, 2006). From these results, it was assumed that the C to N ratio was lower when N, P and K were sufficient. When nitrogen and potassium stressed the microbial population, the C to N ratio increased. This indicated that soil nutrient supply and nutrient balance could influence the C/N ratio of soil microbial biomass, and that this C/N ratio might be an index of soil nutrient status.

Soil biological activity and microbial community structure tested by BIOLOG

The relationship between AWCD and incubation time is shown in Fig.2. In the first 32 h of incubation,

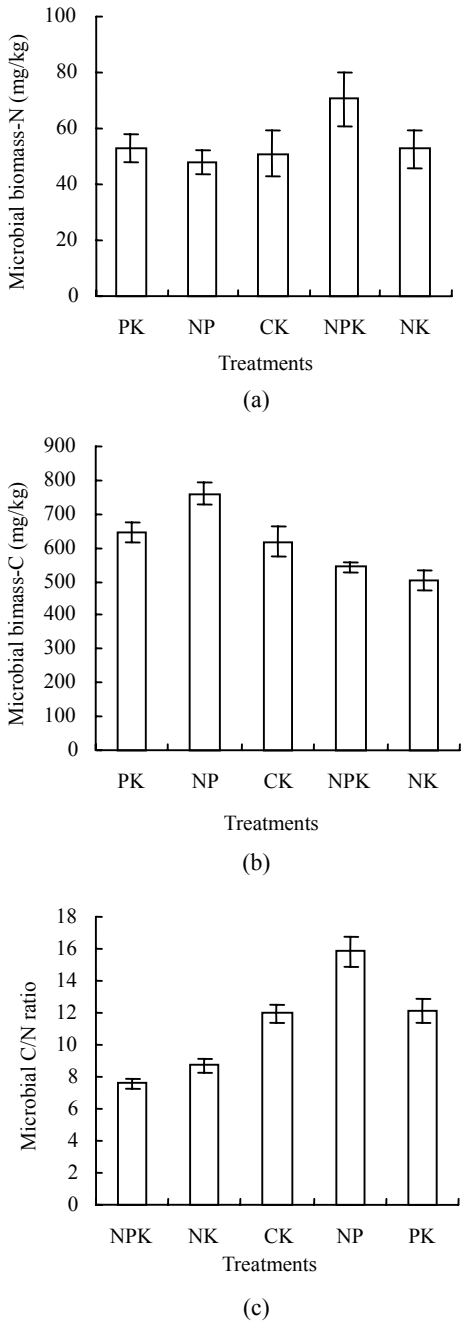

Fig.1 Soil microbial biomass carbon (a), nitrogen (b) and C/N ratio (c) in different treatments

AWCD changed very little but afterwards AWCD increased continuously. This suggested that C sources had not been used in the first 32 h of incubation. At the same time, AWCD of NPK treatment was the highest in all treatments. AWCD of CK treatment increased gradually and was higher than that of NP, PK and NK treatments at the late stage of incubation. It was shown here that deficiency of the necessary nutrients for plants adversely influenced microbial communities. To further analyze the microbial communities, BIOLOG data after 78 h were subjected to canonical variate analysis (CVA) (Fig.3). CV1 explained 52.20% of variance in the data, and CV2 explained 30.45% of variance in the data. This result showed the structure of microbial community changed under different fertilization treatments. Treatment with balanced nutrients (NPK) significantly increased soil biological activity, and promoted the microbial number utilizing different carbon resources.

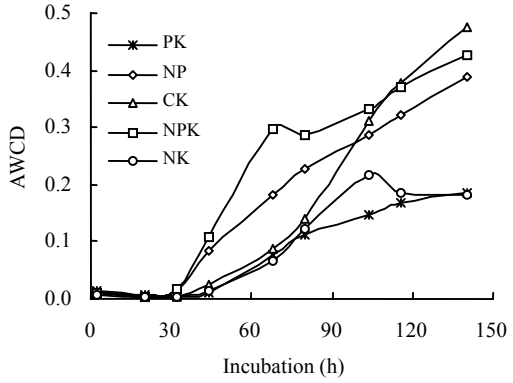

Fig.2 Color development with incubation time. The plot of AWCD represents the mean color response for all 95 response wells

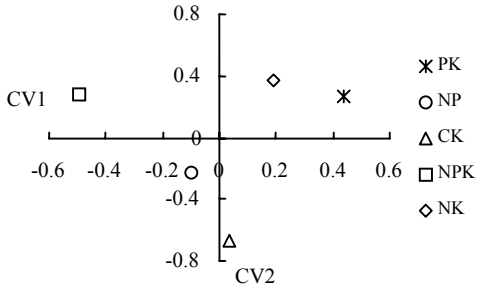

Fig.3 Plot of CV1 and CV2 generated by canonical variate analysis of sole carbon source tests after 78 h in Biolog GN2 plates showing discrimination between different treatments

Soil microbial diversity tested by PLFA

Fig.4 shows that all soil samples contained a variety of PLFAs composed of saturated, unsaturated, methyl-branched and cyclopropane fatty acids. Twenty-nine PLFAs with chain lengths from C12 to C20 were identified by GC, while the PLFAs patterns varied in response to different fertilizer treatments, as revealed by their relative abundance. The

PLFA data were expressed as mol% for multivariate analysis and as $\log_{10}$ moles percent subjected to principal components analysis (PCA). The ordination plot in Fig.5 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 applied N fertilizer treatments (NP, NK and NPK) are found to the right in the plot, with the NPK treatment being in the first quadrant, while the PK treatment was found to the left in the plot. The PK plot showed serious N deficiency after five years consecutive rice cropping in this study site (Zhang and Wang, 2005). This suggested that the different fertilizer practice had an impact on the community structure of specific microbial groups. PCA also identified fatty acids that were important in explaining the variability in PLFA profiles (Fig.6). Specific identified PLFAs, including 15:0, 17:0, 20:0, the branched *i*17:0, the methyl-branched 16:0 (10Me), 18:0 (10Me), and monounsaturated 17:1ω8*c*, as well as cyclopropane *cy*17:0, *cy*19:0 were found on the right in the plot, indicating that these PLFAs increased in applied N treatments, especially in NPK treatment. The PLFAs 16:0, the branched *i*16:0, *a*15:0, and the monounsaturated 16:1ω7, 18:2ω6,9, 18:1ω9, 18:1ω7, were found on the left in the plot (Fig.6). Smithwick *et al*.(2005) found that lipid biomarkers that most likely represent the actionmycetes guild (both 18:0 (10Me) and 16:0 (10Me)) were significantly correlated with gross NH_4^+ mineralization. This study suggested that PLFAs (both 18:0 (10Me) and 16:0 (10Me)) were abundant in applied N treatments, especially NPK treatment.

From the above experimental results, we know that soil nutrient deficiency and unbalance had negative effect on paddy soil microbial biomass and that the diversity of the microbial community, and different fertilizer practice, had impact on the community

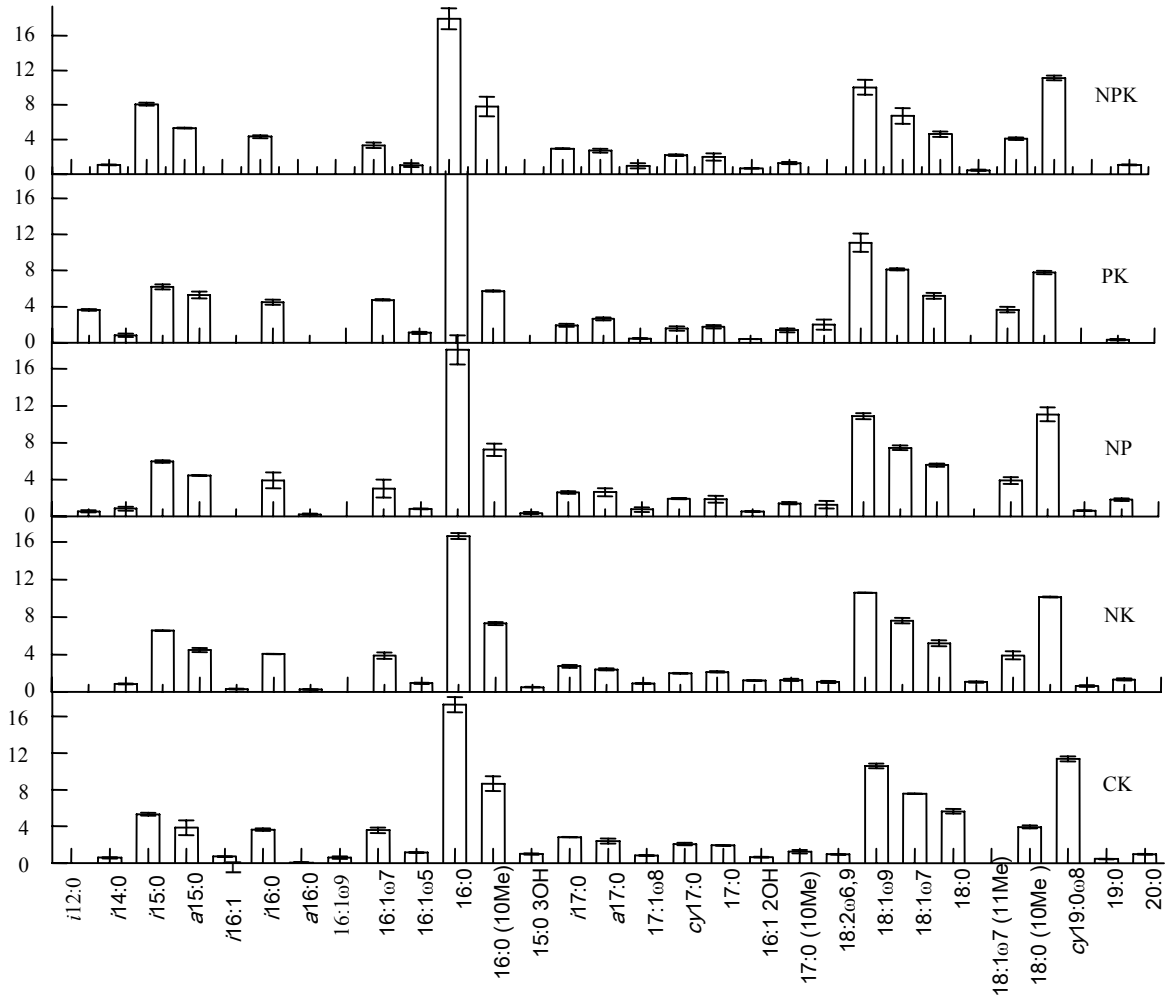

Fig.4 Mol% of different PLFAs in five fertilizer treatments

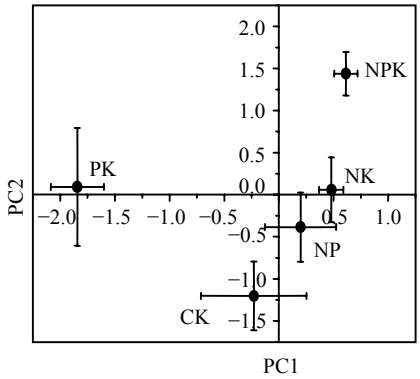

Fig.5 PCA showing variations in PLFA pattern in different fertilizer treatments

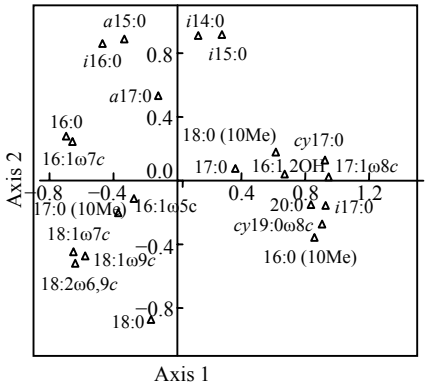

Fig.6 PCA showing loading values for individual PLFAs

structure of specific microbial groups. It seemed that indigenous N supply and N fertilization practice impact on the microbe diversity in soils. There are still a lot to learn regarding microorganisms existing in paddy rice systems. Progress towards this goal will require a better understanding of soil ecology and functional biodiversity.

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