

Evaluating the effect of fertilizer application on soil microbial community structure in rice based cropping system using fatty acid methyl esters (FAME) analysis

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Received: 4 August 2008 / Accepted: 7 January 2009 / Published online: 17 March 2009
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Abstract The present study was undertaken to observe the effects of chemical fertilizers and compost amendments on microbial community structure in rice based cropping system under long term fertilizer application experiment. Changes in microbial community structure assessed by the relative abundance of fatty acid methyl ester (FAME) profiles revealed that compost amendments led to promoting the abundance of Gram-positive bacterial FAMEs. Multivariate analysis of FAME profiles separated the treatments primarily on the basis of the amount of added compost. A link between different fertilizer application treatment and soil microbial components was tentatively established, but it needs to be verified in further studies.

Keywords Microbial diversity · Long term fertilization · Rice · Compost · Fatty acid methyl esters (FAME)

Introduction

Soil microorganisms are involved in various biochemical processes and play a vital role in maintaining soil fertility and plant yields (Nannipieri et al. 2003). Changes in the activity and diversity of soil microbes may reflect changes in soil quality. It has been shown that different soil management practices affected the structure and activity of soil

microorganisms (Patra et al. 2008). In the present scenario maintaining soil microbial activity and diversity have become fundamental aspects in sustainable agriculture. Several reports have indicated that practices associated with organic farming have a positive effect on soil microbial diversity and soil process but there is little evidence of direct negative effects of chemical fertilizers on soil microbial community (Stark et al. 2007). Workers have also advocated establishing the critical cause-and-effect relationship between soil management and soil quality only through long term experiments (Prakash et al. 2007; Stark et al. 2007). The present study was undertaken to determine microbial community structures in a long-term fertilizer application experiment under rice cropping. We have used the analysis of fatty acid methyl ester (FAMEs) to assess variation due to the application of different fertilizer on microbial diversity. This method provides information on the microbial community composition based on groupings of fatty acids (Bossio et al. 1998). Fatty acids make up a relatively constant proportion of the cell biomass and signature fatty acids exist that can differentiate major taxonomic groups within a community. Therefore, a change in the fatty acid profile would represent a change in the microbial population. The analysis of fatty acids extracted from soil has been usefully used previously to study soil microbial community changes in agricultural soils (Bossio et al. 1998; Acosta-Martinez et al. 2008; Patra et al. 2008).

Materials and methods

Study site and soil sampling

The field experiment was established on a Gangseo series (coarse loamy, mixed, non-acid, mesic family of Aquic

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Fluventic Eutrochrepts) paddy soil situated at the National Institute of Agricultural Science and Technology, Suwon city, Gyeonggi-do Province, Republic of Korea. The research plots were established in 1954 to describe the long term effect of different fertilizer amendments on the growth and yield of lowland rice. Compost with and without Nitrogen–Phosphorus–Potassium (NPK) fertilization were selected as treatments. Since 1986 chemical fertilizers have been applied with the rates of $N-P_2O_5-K_2O = 110:70:80 \text{ kg ha}^{-1}$. Compost was added as rice straw at 7.5, 22.5 and 30.0 Mg ha^{-1} in NPKC750, NPKC2250 and NPKC3000 treatments, respectively. The control treatment received neither chemical fertilizer nor compost amendments. Soil samples were collected from the 0 to 20 cm depth using a 1.45 cm diameter soil core at nine randomly selected points in each plot in October 2007 (after harvest). Samples from each treatment were combined to form one composite sample and stored in a sterile polypropylene bag kept cool using coolers during field sampling. After removing visible root debris field moist soil samples were sieved through a 5 mm mesh screen and stored at 4°C in a walk in cooler. Samples were processed within 24–48 h for all analysis.

Microbial community analysis

Fatty acids were extracted from soil samples using the procedure described for pure culture isolates by the Microbial Identification System (MIS, Microbial ID, Newark, DE, USA) as modified for soil analysis (Pankhurst et al. 2001). To prevent the effect of occasionally detected fatty acids, only those fatty acids which account for at least 0.25% of the total content were included in analysis. Fungi were represented by fatty acid 18:2 ω 6,9c, Gram-positive bacteria by 16:0, i15:0, a15:0, i16:0, i17:0, Gram-negative bacteria by 16:1 ω 7c, 16:1 ω 5c, cy17:0 and cy19:0 and total bacteria by the sum of the two previous groups of fatty acids plus 14:0, 15:0, 17:0, a17:0, 17:1 ω 8c and 18:1 ω 11c. The relative abundances of individual FAMES were

calculated as weight percentages (wt%) of total weight ($\mu\text{g g}^{-1}$ soil) of the selected FAMES used to define a microbial group.

Statistical analysis

The data were statistically analysed using SYSTAT 12.0. Treatment means were analysed by one way ANOVA and significant difference among the means were judged by Tukey's post hoc test. Differentiation of the FAME profiles was done by using canonical variates analysis (CVA), after first reducing the dimensionality by principal component analysis (PCA). All CVA and PCA analyses were performed using the Canoco Software for Windows 4.5.

Results and discussion

The effects of different long term fertilizer application treatments on the relative abundances of selected signature FAMES are shown in Table 1. The pattern of FAME profile varied in response to different fertilizer treatments, as revealed by their relative abundances. The sum of all extracted signature fatty acids was highest in NPKC3000 treatment and lowest in control. Relative abundance of fungi and total bacteria were not significantly different in various treatments. The relative abundance of Gram-negative bacteria was highest in control and lowest in NPK treatment. There was no significant difference in the relative abundance of Gram-negative bacteria among compost amended treatments. The relative abundance of Gram-positive bacteria were significantly higher in compost-amended plots as compared to control or NPK treatments. This increase could be attributed to the direct influence of the bacteria in the compost or by the growth promotion effect of compost towards the indigenous *Bacillus* sp. in soil (Chu et al. 2007). Within the compost amendment treatments there was no significant difference within the relative abundance of various groups of fatty acids.

Table 1 Effect of fertilization on the absolute values and relative abundances of selected indicator fatty acids in paddy soils under long term experiment

Treatment	Total fatty acids ($\mu\text{g g}^{-1}$ soil)	Relative abundance of fatty acids (wt%)			
		Fungi	Bacteria	Gram-positive	Gram-negative
Control	29.1a	8.2NS	91.8NS	39.3a	20.5a
NPK	35.3b	7.7NS	92.3NS	40.1a	13.9b
NPKC750	39.3c	10.2NS	89.8NS	47.2b	18.1a
NPKC2250	41.2cd	9.9NS	90.1NS	47.1b	18.2a
NPKC3000	43.3d	10.5NS	89.5NS	48.2b	19.7a

Different letters within a column indicate different levels of significance ($P \leq 0.05$) by Turkey's multiple comparison test

NS Not Significant

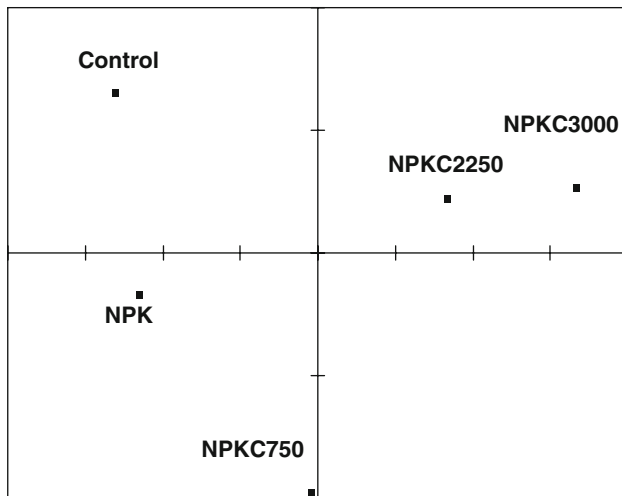


Fig. 1 Canonical variates analysis (CVA) of soil microbial community fatty acid methyl esters (FAME) profiles as affected by different fertilizer application treatments

We used the sensitivity of multivariate analysis to detect treatment differences because it compares the relative abundances of relative fatty acids among treatments. The ordination plot in Fig. 1 shows the differences in the FAME composition of the five different treatments where function 1 (F1) and F2 accounted for 66.92% and 15.85% of the variation, respectively. F1 separated the treatments according to the level of organic amendments. The results suggested that different fertilizer treatments had an impact on the community structure of specific microbial groups. The effects of different fertilizer application and management practices on the microbial community based on FAME profile have been reported (Acosta-Martinez et al. 2008; Patra et al. 2008).

The study has demonstrated that microbial community structure in a rice-based cropping system under long-term experimental changes with different fertilizer application treatments. The amount of FAME was greater in compost amendments. Long term experiments suggested positive effects of compost amendments on microbial diversity which could result in the greater productivity of the cropping system. A link between different fertilizer application

treatments and soil microbial components was tentatively established, but it needs to be verified in further studies.

Acknowledgments Rural Development Authority (RDA), Republic of Korea, is thanked for allowing soil sample collection from experimental plots. Md Rashedul Islam and Pitachi Palaniappan acknowledge Korea Research Foundation (KRF) and Brain Korea (BK), respectively for awarding PhD fellowship.

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