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RESPONSES OF SOIL MICROBIAL COMMUNITY TO PHOSPHATE ROCK AND ANNUAL RYEGRASS (*LOLIUM MULTIFLORUM* LAM.)

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Abstract

An experiment was conducted in a paddy soil to investigate the responses of soil microbial community to a Chinese local phosphate rock sampled from Kunyang, Yunnan Province (KPR) application and annual ryegrass by using phospholipid fatty acid (PLFA) analysis. Results showed that ryegrass planting induced the symbiosis of vesicular-arbuscular mycorrhizae indicated by PLFA 20:4. KPR application produced significant (p<0.05) decline of bacterial and actinomycetic biomass indicated by PLFAs concentration compared with no P application in the absence of plants. Fungi biomass was significantly increased after KPR application in the presence of plants. Changes of soil microbial community structure after high rate of KPR application in the presence of plants may be caused by the interactions between phosphate rock application and ryegrass.

Introduction

Soil microorganisms are very important active components inhabited in soil, as they participate in almost every chemical transformation taking place in soil and play a vital role in soil fertility as a result of their involvement in the cycle of carbon, nitrogen and phosphorus (Ushio *et al*., 2008).

Fertilization can improve soil nutrient status and increase plant productivity. Furthermore, it also influences the activity, biomass and community structure of soil microorganisms. Numerous studies have been conducted on the impact of nitrogen fertilizer, manure, pesticides and different managements on soil microbial community (Lovell *et al*., 1995; Akhtar *et al*., 2004; Mahmood *et al*., 2005, 2007; Murray *et al*., 2006; Toyota & Kuninaga, 2006; Hammesfahr *et al*., 2008). However, few studies were conducted on the impact of P fertilizer particularly phosphate rock application on composition of the soil microbial community (Rooney & Clipson, 2009).

Plant species also has been identified as a factor influencing the composition of microbial community (Paterson *et al*., 2007; Ushio *et al*., 2008). Plant roots may offer C source for growth of microorganism through release of organic compounds, and thus promote increase of microbial activity (Nguyen, 2003).

Because of its slow-release nature, phosphate rock unlikely water-soluble P fertilizer, it cannot be readily taken up by plants and microorganisms after application (Zapata & Roy, 2004), and thus leads to a net accumulation of total soil P fractions. Plant roots may enhance the dissolution of PR in the rhizosphere by excretion of organic acids, uptake of Ca and P (Bolan *et al*., 1997). The increased P status in the rhizosphere may also influence soil microbial activity.

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The objective of this study was to examine impacts of phosphate rock application and annual ryegrass on microbial community in a paddy soil. We also aimed to investigate difference of soil microbial community between two rates of phosphate rock application.

Materials and Methods

Soil: A surface paddy soil sample (0-15 cm depth) was taken from a long-term site-specific nutrient management trial in Jinhua city of Zhejiang province of China. The paddy soil was developed from alluvial deposit. Soil was air-dried and ground to pass through 2 mm sieve for pot experiment. The properties of the soil were: pH (H_2O) 4.81, total P 255 mg kg^{-1}, Olsen P 4.35 mg kg^{-1} and Fe_{DCB} 2.5 g kg^{-1}. Soil pH was measured in deionized water with a soil/solution ratio of 1:1. Total P was digested with H_2SO_4-$HClO_4$. Available P was extracted by the method of Olsen *et al*., (1954). Total free iron oxide was extracted by the method of Mehra & Jackson (1960).

Phosphorus sources: Kunyang phosphate rock (KPR) was collected from Kunyang, Yunnan Province, China. The sample was ground to pass through 0.149 mm sieve. The properties of the KPR were: pH (1:5H_2O) 7.0, total P 138.5 g kg^{-1}, 2% citric acid extractable P 29.1 g kg^{-1}. KPR mineral was identified by standard X-ray diffraction (Phillips-PW1732 X-diffractometer using nickel filter Cu radiator with intensity of scan at 2° min^{-1} at electric pressure 40 KV and electric current 20 mA). The empirical formula of KPR was $Ca_{9.83}Na_{0.12}Mg_{0.05}(PO_4)_{5.50}(CO_3)_{0.50}F_{2.20}$.

Pot experiment: A pot experiment was conducted in the greenhouse of Zhejiang University with annual ryegrass (winter green) planted and unplanted control in 2008. The unplanted control was used to assess microbial community in the absence of ryegrass plant. P treatments of ryegrass planted (planted) and unplanted (unplanted) both include: no P application (Control), application of KPR 50 mg P kg^{-1} (KPR_{50}) and application of KPR 250 mg P kg^{-1} (KPR_{250}). P was applied once at the beginning of the experiment and closely mixed with soil. The pots were arranged in a randomized complete block design with three replicates. Approximately 30 seeds of ryegrass were sown per pot. After one-week growth, the ryegrasses were thinned to 20 plants per pot. All pots were irrigated with deionized water to maintain 80% soil water-holding capacity during the entire experiment.

The above ground plant components were harvested 70 d after seeding when the plants were 30 cm high. These were then oven dried, weighed and ground to pass through a 2 mm sieve. The concentration of P in the plants was determined by the method of Murphy & Riley (1962) after digestion with H_2SO_4-H_2O_2. Soil samples were taken from each pot after harvest, freeze-dried immediately at -50°C, and then stored at -70°C for microbial community analyses. Root material was removed from sieved soil samples before lipid extraction.

PLFA analysis: Lipid extraction and PLFA analysis were performed by the method of Bossio *et al*., (1998). Three g (dry equivalent weight) was extracted with a chloroform-methanol-citrate butter mixture, and the phospholipids were separated from other lipids on a silica-bonded phase column (SPE-Si, Supelco, Poole, UK). The phospholipid fraction was subjected to mild alkaline hydrolysis for producing fatty acid methyl esters before analysis. *c*19:0 was used as the internal standard. Fatty acids were analyzed by Agilent 6890 gas chromatography with a flame ionization detector carried out by a MIDI Sherlocks microbial identification system (Version 4.5, MIDI, Newark, NJ, USA).

The fatty acid nomenclature used follows: total number of carbon atoms: number of double bonds, followed by the position of the double bond (ω) from the methyl end of the molecule. *Cis* geometry is indicated by the suffixes *c*. The prefixes *a* and *i* refer to anteiso- and iso-branching, 10Me indicates a methyl group on the tenth carbon atom from the carboxyl end of the molecule, and *cy* indicates cyclopropane fatty acids (Bossio *et al*., 2006). Each value represents mean of three replicates.

Bacterial PLFAs are represented by *i*15:0, *a*15:0, 15:0, *i*16:0,16:1ω7*c*, *i*17:0, *a*17:0, *cy*17:0, 17:0, 18:1ω7*c* and *cy*19:0ω8*c* (Bossio *et al*., 1998); fungal PLFAs are represented by18:2ω6,9*c* and 18:3ω6*c* (6,9,12) (Myers *et al*., 2001; Vestal & White, 1989); actinomycetic PLFAs are represented by 16:0 (10Me), 17:0 (10Me), 18:0 (10Me) (Turpeinen *et al*., 2004).

Statistics: Statistical procedures were carried out with SPSS Version 13.0. Analysis of variance (ANOVA) was performed by using Fisher's least significant difference comparison of means (LSD). Principal component analysis (PCA) and cluster analysis were completed using the content of individual fatty acid methyl esters. The PCA and cluster analysis were carried out with the correlation matrix and Ward's method, respectively.

Results

Growth of ryegrass: The pot experiment on the paddy soil showed that two rates of KPR application to ryegrass both led to significant (p<0.05) increases in above ground plant biomass and P uptake (Table 1). The plant growth and P uptake on KPR_{250} treatment were significantly (p<0.05) higher than KPR_{50} treatment.

Amounts of PLFAs: The predominant PLFAs in the six treatments were 16:0, *i*15:0, 18:1ω9*c*, 18:1ω7*c*, *cy*19:0ω8*c*, 16:0 (10Me) and *a*15:0, especially 16:0. Concentrations of PLFAs 16:1ω7*c* and 18:2ω6,9*c* were significantly (p<0.05) higher in KPR_{50} (planted) and KPR_{250}(planted) soil than in Control (planted), Control (unplanted), KPR_{50} (unplanted) and KPR_{250} (unplanted) soil. PLFA 20:4 was only detected in soil with ryegrass planted (Fig. 1).

PLFAs variation of microbial group: PLFAs concentration of three microbial groups in six treatments all followed: bacteria>actinomycete>fungi (Fig. 2). In the presence of plants, fungal biomass was increased significantly (p<0.05) in soils after KPR application compared with no P application, bacterial and actinomycetic biomass were less or not affected by KPR application. In the absence of plants, bacterial and actinomycetic biomass were decreased significantly (p<0.05) in soils with KPR application compared with no P application, and soil fungal biomass did not differ significantly among three P treatments.

Microbial community structure: The PCA of the PLFA composition (Fig. 3) showed that the first two principal components PC1 and PC2 account for 58.95% and 15.49% of the variation, respectively. PLFAs 16:0 (10Me), 17:0 (10Me) and 18:0 (10Me) representing actinomycete, 16:1ω9*c*, *i*15:0, *a*16:0, 14:0, *i*16:0, 18:1ω9*c* and *cy*19:0 were highly positively correlated with PC1. PLFAs 18:2ω6,9*c* and 18:3ω6*c* (6,9,12) representing fungi, 20:4 and 16:1ω7*c* were highly negatively correlated with PC1.PLFAs 18:1ω7*c*, *i*14:0 and 18:2ω6,9*c* were highly positively correlated with PC2. It can be found that no PLFA was highly negatively correlated with PC2.

Table 1. Dry weight of ryegrass and uptake of P in the pot experiment.

Treatment	Dry weight (g pot^{-1})	P uptake (mg pot^{-1})
Control (planted)	0.80 ± 0.02 c	0.86 ± 0.09 c
KPR_{50} (planted)	0.90 ± 0.02 b	1.02 ± 0.10 b
KPR_{250} (planted)	1.10 ± 0.01 a	2.02 ± 0.20 a

Means ±SD with the same letter within the same column are not significantly different at 0.05 level

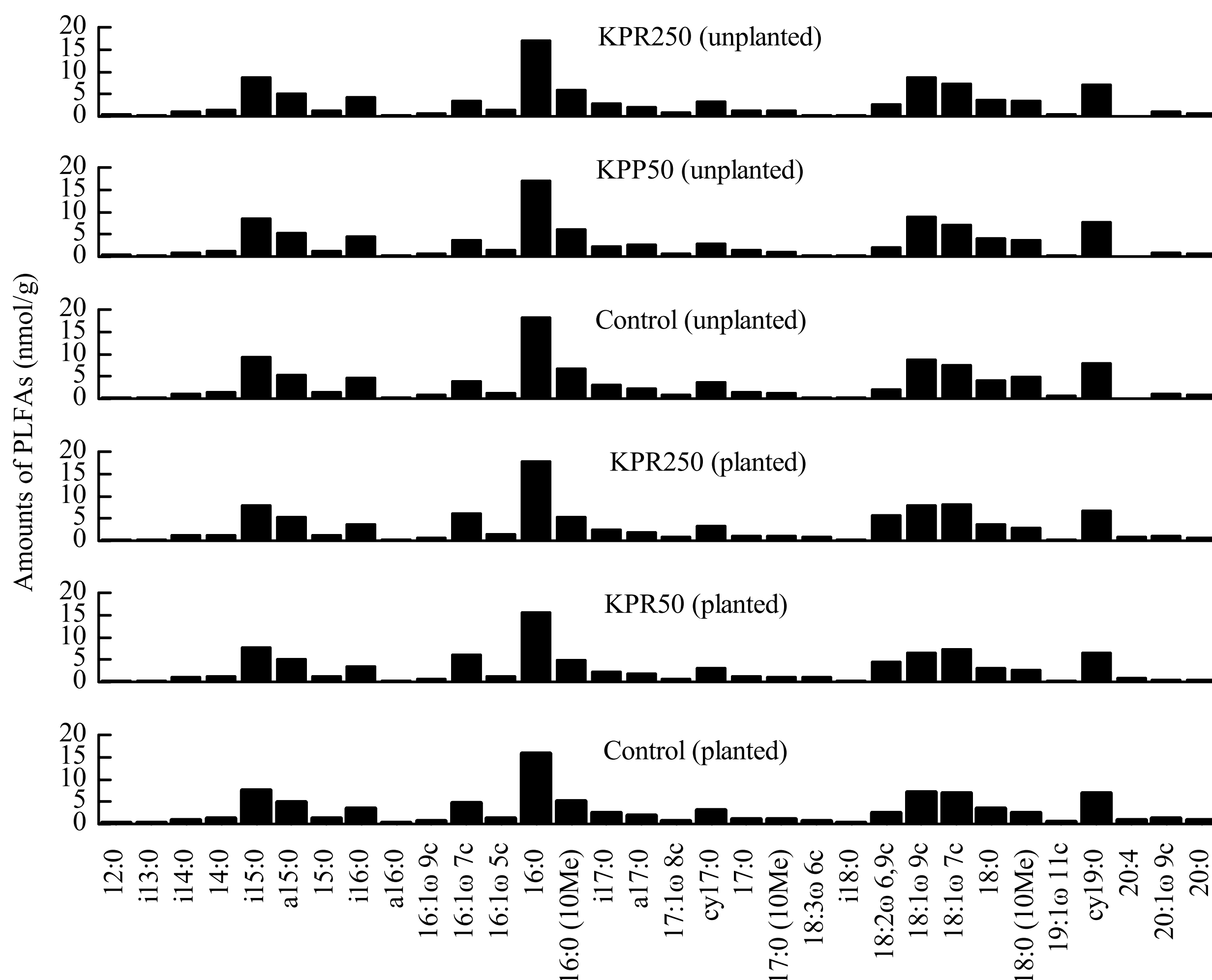

Fig. 1. Content of different PLFAs in Control (planted), KPR_{50}(planted), KPR_{250}(planted), Control (unplanted), KPR_{50}(unplanted) and KPR_{250}(unplanted) treated soil.

P1: 12:0, P2: *i*13:0, P3: *i*14:0, P4: 14:0, P5: *i*15:0, P6: *a*15:0, P7: 15:0, P8: *i*16:0, P9: *a*16:0, P10: 16:1*ω9c*, P11: 16:1*ω7c*, P12: 16:1*ω5c*, P13: 16:0, P14: 16:0 (10Me), P15: *i*17:0, P16: *a*17:0, P17: 17:1*ω8c*, P18: *cy*17:0, P19: 17:0, P20: 17:0 (10Me), P21: 18:3*ω6c*(6,9,12), P22: *i*18:0, P23: 18:2*ω*6,9*c*, P24: 18:1*ω9c*, P25: 18:1*ω7c*, P26: 18:0, P27: 18:0 (10Me), P28: 19:1*ω*11*c*, P29: *cy*19:0*ω8c*, P30: 20:4, P31: 20:1*ω9c*, P32: 20:0.

Cluster analysis of the PLFAs data showed that microbial community structure in the six treatments can be classified into two large clusters (Fig. 4): (1) KPR_{50} (unplanted), KPR_{250} (unplanted) and Control (unplanted), (2) Control (planted), KPR_{50} (unplanted) and KPR_{250} (unplanted). The first cluster was further subdivided into KPR_{50} (unplanted) and KPR_{250} (unplanted); Control (unplanted), respectively. The second cluster was further subdivided into Control (planted) and KPR_{50} (unplanted); KPR_{250} (unplanted), respectively.

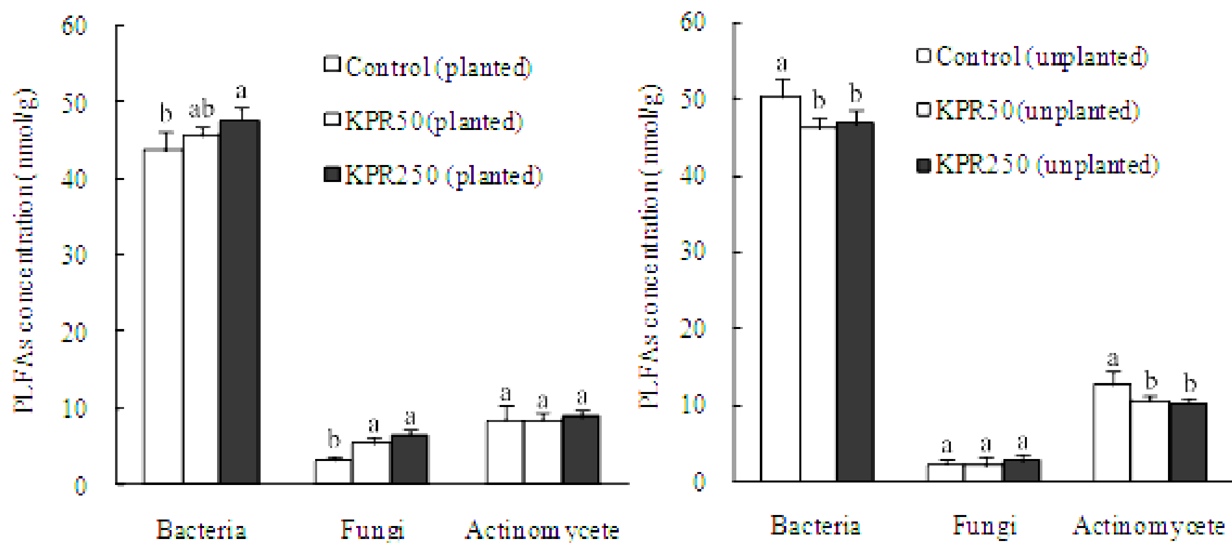

Fig. 2. PLFAs concentration of microbial group in Control (planted), KPR_{50} (planted), KPR_{250} (planted), Control (unplanted), KPR_{50} (unplanted) and KPR_{250} (unplanted) treated soil. Bars are standard deviations.

Means with the same letter within the same column are not significantly different at 0.05 level

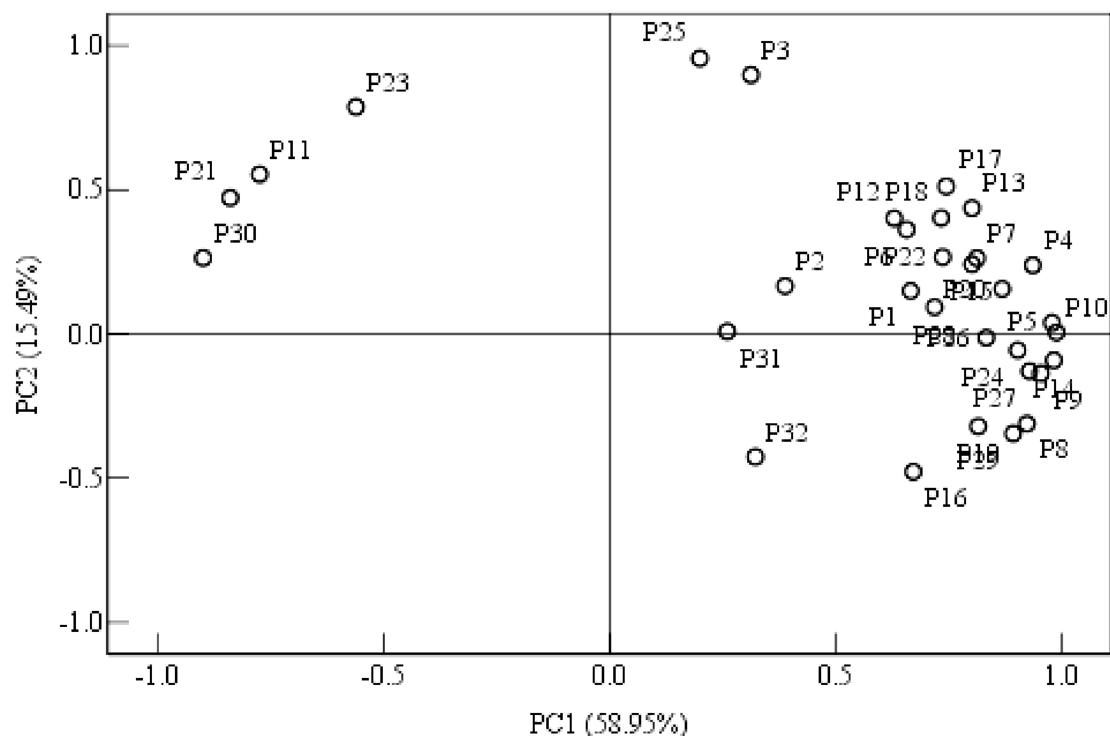

Fig. 3. Principal component analysis of individual PLFA from Control (planted), KPR_{50} (planted), KPR_{250}(planted), Control (unplanted), KPR_{50}(unplanted) and KPR_{250}(unplanted) treated soil.

Discussion

The responses of soil microbial community to phosphate rock application and ryegrass were investigated in a paddy soil with low P status. PLFAs are widely accepted as biomarkers that indicate viable components of soil microbial biomass, and PLFA analysis can provide more detailed information of the "active" soil microbial community compared with the culture method (Vestal & White, 1989; Yao *et al*., 2000; Liang *et al*., 2008). Specific PLFAs also can be used as indicators of taxonomic or functional groups (Bossio *et al*., 1998; Sakamoto *et al*., 2004).

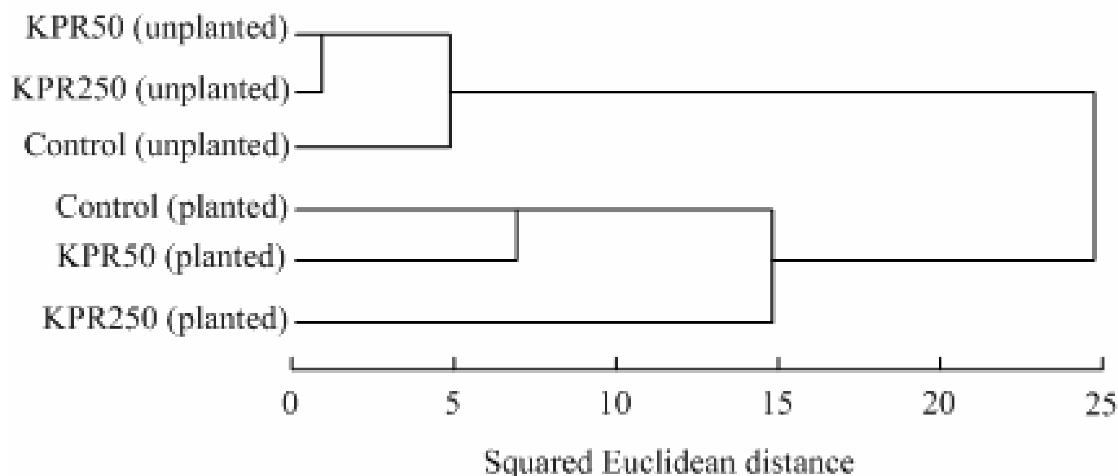

Fig. 4. Cluster analysis of PLFA profile from Control (planted), KPR_{50} (planted), KPR_{250}(planted), Control (unplanted), KPR_{50}(unplanted) and KPR_{250}(unplanted) treated soil.

PLFA 20:4 was found in the spore of *Glomus*, *Gigaspora*, *Scutellospor*a and *Acaulospora* species, in the plant roots colonized by *Glomus* species, and in the external hyphae of two *Glomus* species (Sakamoto *et al*., 2004). All of these species belong to *vesicular-arbuscular mycorrhizae* (VAM). Herbaceous plants with very coarse root systems normally rely on VAM infection to maintain adequate rates of phosphate uptake (Fitter, 1977). In present study, PLFA 20:4 was only found in soils with ryegrass planted. Since soil samples were sieved and roots were taken out before lipid extraction, it is unlikely that PLFA 20:4 comes from plant roots. It seems that ryegrass planting induced the symbiotic association of those types of VAM with plant roots.

Fungi biomass was significantly ($p<0.05$) increased in soil with KPR application compared with no P application in the presence of plants. The paddy soil used in this study was taken from a long-term field experiment with double rice cropping after seven years of no P application, which was low in available P. This was agreed with the conclusion of Rooney & Clipson (2009) that the increase in microbial activity as a result of phosphate addition may reflect P limitations in soil. The significant increase of soil fungi biomass may be explained by increased nutrient availability, enhancement of roots growth and roots exudation stimulate fungi (with a wide range of catabolic pathways) to utilize carbon sources unavailable to other microorganisms (Steer & Harris, 2000).

Shifts in microbial community structure have been attributed to changes in resource availability, particularly root exudates, quantitative and qualitative changes in the input of organic substrates, differences in soil nutrient availability and plant species (Holland & Coleman, 1987; Mawdsley & Bardgett, 1997; Yeates *et al*., 1997; Ushio *et al*., 2008). The factors potentially responsible for driving shifts in soil microbial communities in this study are phosphate rock application, plant species and their interactions.

The results of cluster analysis clearly demonstrate that microbial community in soils with ryegrass planted was different from soils without ryegrass planted. This may be explained by plant species have a significant effect on microbial activity, as exudates and deposits from plant roots are known to offer organic nutrients for microorganisms (Fog, 1988; Bardgett & McAlister, 1999).

It was found that different soil microbial community was established after KPR application compared with no P application in the absence of plants. Microbial communities in soils with two rates of KPR application were similar. This may be attributed to the significant ($p<0.05$) decrease of soil bacterial and actinomycetic biomass after KPR application compared with no P application in the absence of plants.

Different soil microbial community was also established after KPR_{250} application compared with KPR_{50} and no P application in the presence of plants. Phosphate rock is poorly soluble P fertilizer, the contribution of low rate of phosphate rock application to improving P nutrient of soil with low P status is limited (Zapata & Roy, 2004). Such change of microbial community may be caused by the response of ryegrass roots to high rate of phosphate rock application. It was found that the increased nutrient availability might increase C input to soils through enhanced fine root turnover (King *et al*., 2002; Dawson *et al*., 2003), relative utilization of this C source is an important driver of microbial group abundance (Paterson *et al*., 2007). It seems that alteration of microbial community structure in soil with KPR_{250} application after ryegrass planted may come from the interactions between phosphate rock application and ryegrass, and plant induced process may serve as a "starter".

Conclusions

The findings suggested that the interaction between phosphate rock application and ryegrass may affect soil microbial community. The changes in soil microbial community structure after high rate of KPR application in the presence of plants may be caused by the interactions between phosphate rock application and ryegrass.

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