Effects of different organic fertilizers on soil microbial biomass and peanut yield

XJ Lin[A,B], F Wang[A], HS Cai[A], RB Lin[A], CM He[A], QH Li[A] and Y Li[A]

[A]Institute of Soil and Fertilizer, Fujian Academy of Agricultural Sciences, Fuzhou 350013, China,
[B]Corresponding author. Email xinjianlin@163.net

Abstract
A pot experiment was conducted to investigate the effects of different organic fertilizers on soil microbial biomass and peanut yield using plate counting and Denaturing Gradient Gel Electrophoresis (DGGE) methods. The treatments included a) no fertilization (CK), b) chemical fertilizer, c) pig manure, d) cattle manure, e) organic compound fertilizer of monosodium glutamate, and f) chicken manure. Results have shown that both the economic and biological yield of peanut was improved by applying fertilizers, with highest yields being found for applying a compound organic fertilizer of monosodium glutamate. On average, the economic and biological yield in all treatments with applications of either chemical or organic fertilizers increased by 73.5% and 50.0%, respectively, compared with CK. Total amounts of bacteria, epiphyte and actinomyces in the treatment of applying chicken manure were the highest among different fertilizer treatments, while they differed little among other treatments. Results from PCR amplification of soil DNA and DGGE analysis indicated significant differences in soil microbial composition and diversity among different chemical and organic fertilizer treatments. Different organic fertilizers affect the biomass of soil microbes as well as their diversity trait.

Key Words
Peanut, yield, organic fertilizer, soil microbial biomass, DGGE.

Introduction

Application of organic fertilizers is one of important practical measures to improve soil fertility. In addition to providing necessary nutrients for crops and improving soil physico-chemical properties, organic fertilizer is able to enhance soil microbial activity of soil, such as improving activity of soil enzymes and increasing soil microbial biomass (Ren *et al.* 1996; Sun 2003; Lv *et al.* 2005). However, a lot of soil microbes are at nutrition-deficient or un-culturable levels under natural environmental conditions. The measurement of total microbial biomass with rich beef broth in the laboratory will introduce a great error due to the fact that a lot of nutrient-deficient microbes cannot grow. In general, traditional plate culture method can only separate 0.1%~1% of soil microbes present, and cannot reflect the original status of soil microbial diversity (Cai and Liao 2002; Vigdis and Lisc 2002; Luo *et al.* 2003). With the advances in the application of molecular techniques, PCR-based techniques have been widely used (e.g. Bossio *et al.* 2005; Zhang *et al.* 2007). The objective of this study was to examine the effects of application of organic manures on peanut production and the impacts on soil microbial biomass and diversity of soil microbe composition using combined plate counting and PCR-based Denaturing Gradient Gel Electrophoresis (DGGE) methods.

Methods

Soil

A clayey grey-yellow soil collected from Xitou village, Baisha town, Miuhou county was used in this study. Soil pH was 5.3, while total N, P and K were 1.26, 0.27 and 4.3 g/kg respectively. Available N, P and K amounted to 183.1, 39.3 and 25.1 mg/kg respectively. Soil organic matter was 10.4 g/kg. Basic properties of main organic fertilizers were shown in Table 1.

Table 1. Basic properties of organic fertilizers used in this study

Organic fertilizer[a]	OM (g/kg)	Total N (g/kg)	Total P (g/kg)	Total K (g/kg)	pH
Organic compound fertilizer of monosodium glutamate	180	105.0	13.0	30.0	6.1
Pig manure	500	8.8	26.0	9.5	8.7
Cattle manure	410	13.0	9.0	11.0	6.9
Chicken manure	410	29.3	36.8	18.7	8.3

Treatments
The experiment included six treatments with four replications and fully-randomized arrangements. The treatments were: a) no fertilization (CK); b) chemical fertilizer; c) pig manure; d) cattle manure; e) organic compound fertilizer of monosodium glutamate; and f) chicken manure. Plastic pots (26 x 22.5 x 29 cm) were used for this pot experiment. Each pot contained 7.5 kg soil, for each kg soil, 0.1g N was supplied, and $N:P_2O_5:K_2O$ ratios were 5:4:6. The equivalent N, P and K were given to each treatment. The corresponding amounts of chemical fertilizers (carbamide, potassium chloride, potassium dihydrogen phosphate) were supplemented to the treatments of organic manures to make up the deficient part of N, P and K. Fertilizers were fully mixed with soil on the plastic sheet, and then loaded into pots. Seven peanuts were planted, but only three seedlings were finally selected. Plants were harvested and yield of peanuts were measured four months after planting.

DGGE Analysis of soil microbial diversity
Total DNA of soil microbe was amplified by using 16s rDNA V3 primers that No.1 is 5-ATTACCGCGGCTGCTGG-3, No.2 is 5-(GC)-CCTACGGGAGGCAGCAG-3 (Zhao *et al.* 2005). The reaction system was that 10×amplified buffer solution 2.5μL of 25 mM $MgCl_2$. 2μL of 2.5 mM dNTP, No.1 primer and No.2 primer was 0.5μL, respectively. The template DNA was 20-40 ng, add water to 24.5 μL of Taq enzyme was 0.5 μL (5U/μL) and the total volume is 25 μL. The procedure for amplification was denaturalized at 95 °C for 5min, 94°C for 1min, 50 °C for 1min, 72 °C for 40s, 30 circulations, and then extended 10min at 72 °C,and stored at 4 °C. DGGE was carried out on the amplified outcome of 16S rDNA (V3 selection) by Dcode universal mutation detection system (Bio-Rad). Denaturant concentration is prepared from 30% to 70% with 8% polyacrylamide gel electrophoresis, the denaturant concentration increased from upper to underside. When the gel solidified, it was pre-heated to 60 °C. 20μL PCR amplified outcome and 2×loading buffer(70% butter,0.05% bromophenol blue, 0.05% dimethylbenzene) were loaded into each hole, and electrophoreses was done for 16h at 70V. The gel was dyed 30min with 1000 times dilution of syber green after electrophoresis. The dyed gel is observed and pictured through imaging system (Bio-Rad).

Results
Effect of different treatments on peanut yield
Table 2 has shown that peanut legume yields increased significantly (by 42.8% - 73.5%) in all fertilizer treatments, compared with CK. The order of legume yield was: organic compound fertilizer of monosodium glutamate > pig manure > cattle manure > chemical fertilizer > chicken manure. Total peanut biomass also increased significantly (28.3-50.0%) in all fertilizer treatments compared with CK, with the order: organic compound fertilizer of monosodium glutamate >chemical fertilizer>pig manure>chicken manure >cattle manure (Table 2).

Table 2. Effects of different fertilizer treatments on the yield of peanut[a]

Treatment	Legume yield (g/basin)	Increase than CK (%)	Biological yield (g/basin)	Increase than CK (%)
CK	7.2±1.11b	-	18.9±1.46c	-
Inorganic fertilizer	10.5±1.25a	45.17	28.1±3.31ab	48.41
Pig manure	11.3±2.92a	55.52	27.0±4.73 ab	42.72
Cattle manure	10.8±1.06 a	49.31	24.3±0.66b	28.31
Organic compound fertilizer of monosodium glutamate	12.6±0.90 a	73.45	28.4±2.25a	50.00
Chicken manure	10.4±1.30 a	42.76	25.6±0.92ab	35.32

[a]Data followed by the same letter are not significant (P >0.05).

Effect of different treatments on soil microbial biomass using plant count method
Except organic compound fertilizer of monosodium glutamate, all fertilizer treatments increased total bacterial counts compared with CK, with a biggest increase in the chicken manure treatment (by 433%) (Table 3). All fertilizer treatments increased fungi counts except for the cattle manure treatment, also with a biggest increase in the chicken manure treatment (717%) (Table 3). Soil actinomyces increased in population compared with CK in all treatments except for the chemical fertilizer treatment.

Effect of different treatments on soil microbial community composition using DGGC
The amplified products of soil total microbe DNA16SrDNA V3 of different fertilizer treatments were

analyzed by Denatured Gradient Gel Electrophoresis (DGGE) (Figure 1). The DGGE bands of DNA samples from different fertilizer treatments could be well separated, where the intensity and transferring rates were not the same. Meanwhile, many common bands were found in different treatments, but the intensity was different. There were deeper colour bands to compare to other samples when samples were from the treatments applying pig manure, chicken manure, and organic compound fertilizer of monosodium glutamate. It demonstrated that using all kinds of organic fertilizers promoted the increase of some microbial biomass to compare with the treatment of CK and chemical fertilizer. The band patterns appeared different when different organic fertilizers were applied to the soil. For example, dark colour band in treatment of pig manure appears No.1 strip, but in that of organic compound fertilizer of monosodium glutamate appears both No.3 and No.4. Maybe, the effects of soil microbial diversity have been affected by some special substance in pig manure or organic compound fertilizer of monosodium glutamate. However, the correlated degree needs to be further investigated. The band patterns were similar in the treatment of applying chicken manure and organic compound fertilizer of monosodium glutamate (e.g. band No.3 and band No.4). The treatment of chicken manure also has these two bands, but the concentration is low. It showed that both chicken manure and organic compound fertilizer of monosodium glutamate may contain some substance that promotes microbial propagation.

Table 3. Effects of different fertilization on microbial community

Treatment	bacteria (unit/g)	Epiphyte (unit/g)	Actinomyces (unit/g)
CK	5.35×10^6	2.40×10^5	6.60×10^5
Inorganic fertilizer	6.10×10^6	4.00×10^5	5.70×10^5
Pig manure	6.40×10^6	5.95×10^5	1.08×10^6
Cattle manure	5.40×10^6	2.20×10^5	8.15×10^5
Organic compound fertilizer of monosodium glutamate	3.80×10^6	5.95×10^5	8.05×10^5
Chicken manure	2.85×10^7	1.95×10^6	3.95×10^6

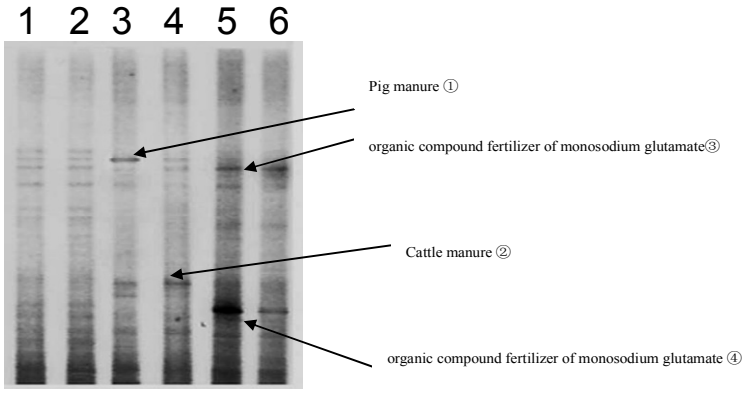

Figure 1. Soil DGGE pattern of different fertilization treatments. M shows λDNA／HindIII marker, lane 1-6 show:1 CK；2 Inorganic fertilizer；3 Pig manure；4 Cattle manure；5 Organic compound fertilizer of monosodium glutamate；6 Chicken manure.

Conclusions

All fertilizer treatments increased both peanut legume yield and biomass compared no fertilization (CK), with the higher increment in the treatments of monosodium glutamate. Plate count and DGGE analysis have demonstrated that application of organic manure substantially increased soil microbial biomass and microbial community (species) diversity.

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