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Full Length Research Paper

Variation of soil enzyme activities and microbial community structure in peanut monocropping system in subtropical China

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The changes in soil microbial community are supposed to be one of key factors for peanut yield decline in long-term continuous monocropping systems. A series of peanut fields, where peanut were continuously monocropped for 3, 6, 10 and 15 years in subtropical China, were selected to investigate the effect of continuous monocropping on peanut yield, soil microbial community structure and enzyme activity. Peanut yield, urease and invertase activities decreased with time, but the activity of polyphenol oxidase decreased in the first several years and then increased. The results of both culture dependent methods and phospholipid fatty acids (PLFA) analysis showed that bacteria (Gram-positive and Gram-negative bacteria) and actinomycetes decreased, while fungal colony-forming units (CFUs) increased with time. Canonical Correspondence Analysis (CCA) showed that with increasing peanut monocropping years, soil microbial structure became more correlative with fungi compositions. The proportion of bacteria in total PLFA decreased from 67.4% to 53.0%, meanwhile the proportion of fungi was increased from 16.9% to 32.8%. Denaturing gradient gel electrophoresis (DGGE) analysis indicated that bacteria diversity decreased and fungi diversity increased with time, and changes in fungi diversity were much greater than those of bacteria.

Key words: DGGE, enzyme activities, monocropping, peanut, PLFA, red soil.

INTRODUCTION

Peanut (*Arachis hypogaea L.*) is an important oilseed crop cultivated widely in tropical, subtropical and warm temperate climate regions around the world. China is one of the largest peanut producers with a peanut planting area of more than 40 km^2 in 2008 (Ministry of Agriculture of the People's Republic of China, 2009). Peanut was generally monocropped on a large scale and continuously for long periods in the same field in some regions due to limited arable land and a requirement for intensifying regional agro-industrialization (Lian et al., 2010).

Changes in soil microbial community in continuous monocropping system is believed to be responsible for the decline in peanut yield (Sun et al., 2001; Xu and Wang, 2003). Specifically, continuous peanut monocropping may reduce the species and quantity of bacteria and actinomycete, lower the number of fungi species and increase mould quantity (Sun et al., 2001). Similar phenomena have been found in many other crops (Hu et

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al., 2006; Li et al., 2005; Ryszkowski et al., 1998; Xu and Wang, 2003).

The changes in peanut yield and soil microbial community in continuous peanut monocropping field in Fluvo-aquic soil (Chao soil in Chinese) in warm temperate region of North China was generally obtained from 3 to 5 years short-term plot or pot control experiments (Feng et al., 1999; Sun et al., 2001; Wu et al., 2006; Zhang et al., 1992), and the variation of culturable soil microbial community induced by continuous peanut monocropping was studied in detail with the dilution plate method (Feng et al., 1999; Sun et al., 2001). As a matter of fact, the percentage of culturable microbes in soil total microbes is less than 10%. In order to explain the effect of continuous cropping on soil microbial communities more comprehensively, soil microbes that can't be cultured needed to be further researched through culture-independent methods. Denaturing gradient gel electrophoresis (DGGE) is a novel molecular technique that provides broad information on soil microbial species composition based on DNA, which exist in all types of soil microbes, no matter culturable or non-culturable. However, they also have some problems in the reproducibility of nucleic acid extraction and in the selectivity of the PCR step. This has been overcomed by the determination of phospholipids fatty acid (PLFA) profiles, which provide a broad diversity measurement of microbial community at the phnotypic level.

In some fields in the red soil region of subtropical China, peanuts were monocropped for over 20 years. Continuous monocropping obstacles should be more seriously considered in the region (Wang and Chen, 2005; Chen et al., 2008). Wang and Chen (2005) investigated peanut yield change with increasing monocropping years at farm scale and reported that peanut yield in the continuous 10 and 21 years monocropping fields decreased by 28.9 and 51.2%, respectively, compared with the continuous 3 years monocropping field. In general, the distribution and abundance of the soil microbial communities is a function of abiotic (physical and chemical) conditions and biotic factors (interactions among species/ food supply) (Scherer-Lorenzen et al., 2005). The activity of soil microbial communities determines the productivity and overall quality of agricultural ecosystem due to soil microorganisms' role in nutrient cycling, detoxification processes and soil aggregate stability, among other functions (Marcela et al., 2011). However, the change in microbial community structure in the soils after continuous peanut monocropping has not been systematically researched in subtropical China, particularly for the non-culturable microbial community. Our previous studies indicated that *p*-hydroxybenzcic acid, vanillic acid and *p*-coumaric acid as important autotoxic substances of peanut are accumulated in the soil in continuous monocropping systems, and thereby inhibiting peanut growth. The phenolic acids can also increase the incidence of fungi disease in peanut seedling and reduce the activities of antioxidation enzymes in peanut seedling (Li et al., 2010). The objectives of this study were to investigate the impact of continuous peanut monocropping on peanut yield, soil chemical properties, soil enzyme activities and microbial community structure at farm scale in subtropical China, particularly for soil microbial community changes using dilution plate methods, together with denaturing gradient gel electrophoresis (DGGE) and phospholipid fatty acid (PLFA).

MATERIALS AND METHODS

Experimental design

The research was conducted around the Ecological Experimental Station of Red Soil, Chinese Academy of Sciences, Yujiang, Jiangxi province, China (28°13′N and 116°55′E, altitude 45 m a.s.l). The soil is generally called red soil in Chinese and classified as Udic Ferrosol (Alumi-Orthic Acrisol in FAO taxonomy; Udic Kandiudults in USDA taxonomy). A series of peanut field at same slope upland within 0.5 km^2 were selected to measure peanut pod yield at the beginning of August, 2008 and investigate cropping sequences systems and field management practices in the autumn. Before peanut sowing in 2009, soil samples were collected from four adjacent peanut field sites where peanut were continuously monocropped for 3 (CC3), 6 (CC6), 10 (CC10) and 15 years (CC15), respectively, since the breed of peanut was the same (Ganhua 5), the area of peanut cropping was more than 0.33 hm^2, the fertilization and other field managements were very similar in each sampling field site. Briefly, at the beginning of reclamation for crop planting, 2250 kg hm^{-2} lime was generally broadcasted, and then no more lime was applied in the fields. Peanuts were sown at the beginning of April and harvested at the beginning of August every year. Before peanut sowing, about 300 kg hm^{-2} of urea, 750 kg hm^{-2} of calcium magnesium phosphate, 225 kg hm^{-2} of potassium chlorate and 15 kg hm^{-2} borax were applied along a furrow with 10 cm in width and 10 cm in depth, then peanut sowed in the opposite to fertilizers along the furrow, and then peanut seeds and fertilizers were covered with surface soil. The control (CK) was on abandoned land at the same slope upland, where peanut has not been planted in the last five years. Each field of CK, CC3, CC6, CC10 and CC15 was divided into five sub-sampling plots according to micro-landform characteristics, and top soil samples (0 - 20 cm) were collected at five random points in each sub-sampling plots on March 20, 2009 for one sample. The soil samples were transported to the laboratory on ice. After removing visible sundries, a portion of fresh moist soil from each sample was stored at -20°C for PLFA and DGGE analyses, and another portion was stored at 4°C for the assays of culturable soil microbes. The rest of the samples were air-dried for the assays of soil enzyme activities and agro-chemical analyses. Each sub-sample was used in all these measurements in triplicate.

Peanut yield measurement

Five sub-sampling plots with 0.5 m in width and 2.0 m in length in the corresponding soil sub-sampling plots were selected to measure peanut pod yield in each sampling field site. Peanut pods each sub-sampling plots were collected, cleaned. The biomass was analyzed after drying 80°C for 48 h.

Chemical analysis

Air-dried soil sample was use for chemical analysis according to the

methods suggested by Soil Science Society of China (Lu, 2000). Briefly, pH was measured with a 1:2.5 (w:v) ratio of soil to deionized water using a pH meter. Organic carbon was oxidized by the solution of 0.133 mol L^{-1} $K_2Cr_2O_7$–18.4 mol L^{-1} H_2SO_4 in an oil bath and then the excess $K_2Cr_2O_7$ was titrated with 0.2 mol L^{-1} $FeSO_4$, and thus organic matter content was obtained by multiplying the carbon value by a factor of 1.72. Total N measured by the Semimicro-Kjeldahl methods after soil digested with $HClO_4$ and HF, and total P by molybdenum-blue colorimetry method after soil digested with H_2SO_4 and $HClO_4$. Alkali-hydrolyzable N was released and transformed to NH_3 by 1.0 mol L^{-1} NaOH and $FeSO_4$ powder at 40°C for 24 h, and then absorbed with 2% H_3BO_3 and titrated with 0.005 mol L^{-1} H_2SO_4. Available P extracted with 0.5 mol L^{-1} $NaHCO_3$ and measured by molybdenum-blue colorimetry method. Available K was extracted with 1.0 mol L^{-1} NH_4OAc (pH 7.0) and then determined by flame photometry method.

Enzyme activities measurement

To determine biological function changes in soil, some enzyme activities were immediately determined after soil air-dried (<1 mm) according to the literatures (Institute of Soil Science, Chinese Academy of Sciences, 1985; Martinez et al., 2008). Briefly, soil urease activity was measured by incubating 10.0 g soil with 10 ml of 10% urea solution for 24 h at 37°C. The formation of ammonium was determined spectrophotometrically at 578 nm and the activity was expressed as NH_4–N mg g^{-1} soil 24 h^{-1} (Hoffmann and Teicher , 1961). Soil invertase activity was measured by incubating 5.0 g soil with 15 ml of 8% sucrose solution for 24 h at 37°C. The suspension reacted with 3, 5-dinitrosalicylic acid and absorbance was detected at 508 nm. Activity was expressed as glucose mg g^{-1} soil 24 h^{-1} (Frankenberger et al., 1983). Polyphenol oxidase (EC 1.10.3.1) activity was determined as described by Peruccia et al. (2000), and expressed as purpurogallin mg g^{-1} soil h^{-1}. All determinations of each sample were performed in triplicate, and all values reported are averages of the three determinations expressed on an oven-dried soil basis.

Cultivable microbial analysis

The total numbers of cultivable soil bacteria, fungi and actinomyces were determined from colony-forming-units (CFUs) counted on agar plates using dilution plate methods. Briefly, triplicate 10 g of each fresh sample were homogenized in 90 ml of sterilized water and serially diluted. Aliquots (0.1 ml) of the diluted suspension were spread on the beef extract peptone medium, Cause's No.1 synthetic medium and Rose bengal medium for the enumeration of bacteria, actinomyces and fungi, respectively (Xu and Zheng, 1986). The CFUs on bacterial and actinomyces medium were counted after incubation at 37°C, and for fungi was 28°C. Plates that carried between 50 to 200 colonies were counted on day 1 and day 3 (Institute of Soil Science, Chinese Academy of Sciences, 1985).

PLFA profiles

Microbial biomass was estimated by total phospholipid fatty acids (PLFA), and PLFA profiles were analyzed for assessing the microbial community composition. The PLFA analysis was performed according to the method by Bartelt-Ryser et al. (2005). Briefly, 5.0 g freeze dried soil sample was extracted using a single-phase chloroform–methanol–phosphate buffer (0.1 M pH 7.0) (1:2:0.8, v:v:v) in the dark at room temperature by shaking the tubes horizontally. The polar PLFA fractions were isolated using silica gel chromatograph. The polar lipids were subjected to saponification and methylation by a mild alkaline methanolysis (Guckert et al., 1985). The fatty acid methyl esters (FAMEs) were analyzed using a Hewlett-Packard 1890 capillary gas chromatographer equipped with flame ionization detector and a 50-m polar column. Individual fatty acids were identified according to standards such as 37 fatty acid methyl ester (FAME) mixtures (FAME 37 47885-u, Supelco, Inc.), 24 bacterial FAME mixtures (P-BAME 24 47080-U, Supelco, Inc.), and a number of single fatty acid methyl esters (Lipidox, Sweden). All peak areas were quantified based on the internal standard, methyl nonadecanoate fatty acid (19:0) (Sigma). The fatty acid nomenclature as described by Frostegård et al. (1993) was used in the present study. The PLFAs 15:0, i15:0, a15:0, i16:0 and i17:0 were designated as Gram-positive (GP) bacteria and cy17:0, cy19:0, 16:1ω9 and 18:1ω7 were designated as Gram-negative (GN) bacterial. 18:2ω6, 9c, 18:2ω6 and 9t were used as indicators of fungi, while 10Me16:0 and 10Me18:0 represented soil action-mycetes (Frostegård and Bååth, 1996; Zelles, 1999). The contents of total PLFAs were used as an index of the total viable microbial biomass and the ratio of the sum of bacteria to fungi (B/F) was represented by bacterial PLFAs /18:2ω6, 9c, 18:2ω6, 9c t.

Molecular microbial analysis

The total soil DNA was extracted by the UltraClean Soil DNA kit (Mo Bio Labs) according to the manufacturer's instruction. The bacterial 16S rDNA was amplified using a universal forward primer (Muyzer et al., 1993) F357-GCclamp (5'-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG-3') and a reverse primer 517R (5'-ATT ACC GCG GCT GCT GG-3'). Fungal 18S rDNA was amplified using forward primer (Borneman and Hartin, 2000) EF4-GCclamp (5'-CGC CCG CGC CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GGG AAG GGR TGT ATT TAT TAG-3') and reverse primer Fung5R (5'-GTA AAA GTC CTG GTT CCC C-3').

Touchdown PCR was performed in a total volume of 50 µl using following protocol: initial denaturation at 94°C for 4 min; followed by 8 touchdown cycles of denaturation at 94°C for 40 s; annealing at 58°C (-1°C per cycle) for bacterium (57°C for fungi) for 1.5 min, and extension at 72°C for 2 min; followed by 26 cycles of 94°C for 40 s, annealing at 52°C for 1.5 min and extension at 72°C for 2 min; a final extension at 72°C for 10 min.

The presence of successfully amplified 250 bp (bacteria bands) and 500 bp (fungal bands) PCR products was confirmed by analyzing 3 µl of the PCR product on 1.5% agarose gel, stained with ethidium bromide (EB) and visualized using LEL DOC-II (USA). To avoid the bias of one soil sample, each soil sample had 6 duplications of PCR products. After tested on electrophoresis, the 6 copies were mixed, and concentrated by ethanol precipitation.

For DGGE, 20 µl of concentrated solution of PCR product was used. The DGGE was performed with 7.5% acrylamide gels with a gradient ranging from 40 to 70% for bacterium and 30~60% for fungi (100% denaturant was defined as 7 mol L^{-1} urea and 40% formamide) and at a constant voltage of 110 V and 60°C for 16 h (C.B.S-2001 system, USA). After electrophoresis, the gel was stained with Synergy Brands (SYBR) Green I nucleic acid gel stain (Invitrogen, 1:10,000 dilution) for 30 min, and images were captured using the LEL DOC- II (USA).

Statistical analysis

Basic statistical analyses were performed using SPSS 13.0. One-way ANOVA followed by Tukey's HSD test for determining significant differences between relative data sets. The PLFA data analyses were subjected to a Canonical Correspondence Analysis (CCA). Gel-Compar II (Applied Maths, Belgium) was used for the cluster analysis of soil bacteria and fungi based on the DGGE results.

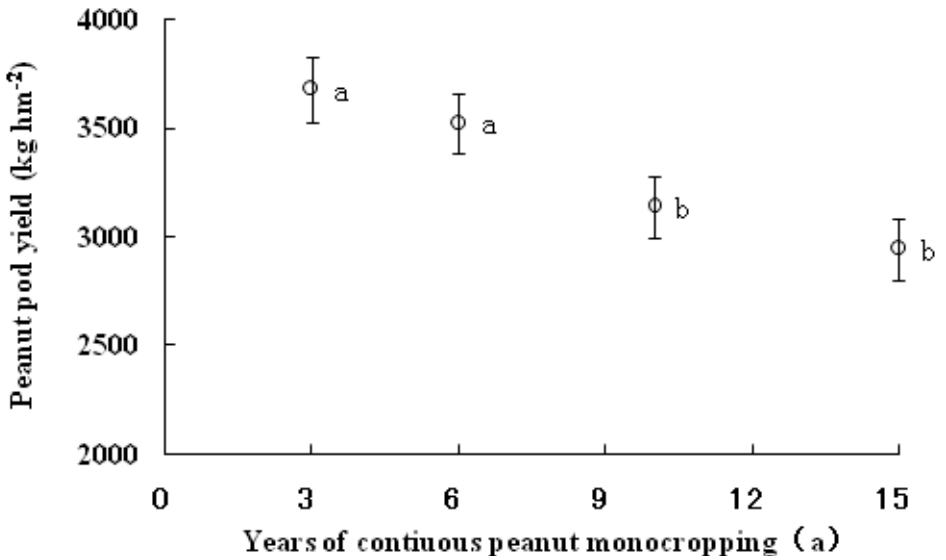

Figure 1. Changes in peanut pod yield with increasing continuous peanut monocropping years. Dot followed by the different letters are significantly different (P<0.05).

Table 1. Basic agro-chemical properties of the soil with different peanut monocropping years.

	pH (H_2O)	Organic matter (g kg^{-1})	Total N (g kg^{-1})	Total P (g kg^{-1})	Alkali-hydrolyzable N (mg kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})
CK	6.42±0.08a	11.5±0.10d	0.55±0.02b	0.31±0.01d	46.2±2.2b	10.4±0.1d	70.0±4.2e
CC3	5.36±0.04b	12.7±0.12b	0.81±0.03a	0.77±0.02a	56.1±4.6a	43.8±3.2ab	95.0±3.2d
CC6	4.71±0.03c	9.93±0.08e	0.63±0.02b	0.30±0.01d	42.9±3.9b	17.0±1.1c	177±9.0c
CC10	4.45±0.02d	13.8±0.15a	0.80±0.06a	0.56±0.02b	62.7±5.4a	40.5±3.9b	355±10a
CC15	4.40±0.02d	11.9±0.09c	0.78±0.09a	0.39±0.01c	59.4±4.3a	47.8±4.5a	327±10b

CK stands for the control. CC3, CC6, CC10 and CC15 stand for continuous peanut monocropping for 3, 6, 10 and 15 years, respectively. Values followed by the different letters are significantly different within columns (P<0.05).

RESULTS

Peanut pod yield

Highest pod yield was found after 3 years of mono-cropping (Figure 1). Pod yield decreased by 4.6, 14.7 and 20.0% after 6, 10, and 15 years of monocropping, compared to the 3 years values.

Agro-chemical properties of soils

Compared with the control, total N, available P and available K in the soil increased after peanut cropped (Table 1), however there no correlation to the time of monocropping, except for pH, which decreased with time from 6.4 to 4.4.

Soil enzyme activity

Soil invertase and urease activities significantly decreased with increasing number of monocropping years (Table 2). After continuous monocropping for 15 years, invertase activity decreased by 91.8%, and urease activity decreased by 40.3%, compared with the control. Polyphenol oxidase activity was highest in the soil of peanut upland with 3-year continuous monocropping, but was lowest at the 6th year of continuous monocropping.

Culturable soil microorganism

The different peanut monocropping treatments caused a shift of the culturable bacterial, actinomycetic and fungal CFUs (Table 3). The largest bacterial CFUs were observed in the CC3 treatment, and the lowest CFUs were found in the CC15 treatment. Actinomycetic CFUs exhibits the similar tendency with bacterial CFUs, which reached to the largest in CC3 and decreased to the lowest in CC15. On the contrary, fungal CFUs were the lowest in CC3 and reached to the largest in CC15. With increasing continuous peanut monocropping years, bacterial and

Table 2. Effect of continuous peanut monocropping on soil enzyme activities.

	Invertase (U)	Urease (U)	Polyphenol oxidase (U)
CK	4.57±0.62 a	0.44±0.02 a	2.80±0.22 b
CC3	4.81±0.80 a	0.32±0.02 b	4.26±0.36 a
CC6	1.36±0.26 b	0.27±0.01 c	0.28±0.10 d
CC10	1.16±0.45 b	0.26±0.01 c	0.42±0.14 d
CC15	0.38±0.13 c	0.26±0.01 c	2.00±0.46 c

Values followed by the different letters are significantly different within columns (P<0.05).

Table 3. Effects of continuous peanut monocropping on soil microbial communities by dilution plate methods.

	Fungi ($\times10^4$CFU·g^{-1} DM)	Actinomycete ($\times10^5$CFU·g^{-1} DM)	Bacteria ($\times10^6$CFU·g^{-1} DM)
CK	10.8±0.76a	52.0±0.50c	22.5±0.50b
CC3	18.3±3.54b	88.3±0.90d	36.2±2.46d
CC6	29.6±1.01c	31.0±2.92b	23.0±2.62b
CC10	32.5±2.09c	29.4±1.46ab	22.4±3.94b
CC15	39.9±1.87d	26.0±1.30a	13.7±3.47a

Values followed by the different letters are significantly different within columns (P<0.05).

actinomycetic CFUs significantly decreased, while fungal CFUs significantly increased.

Soil microbial community analysis based on PLFA

Continuous peanut monocropping significantly affected PLFAs of the microbial community (Table 4). Among the soil microbial community, the proportion of bacteria in total PLFA was from 53.0% to 67.4%, much more than other types of microbes. Bacteria (Gram-positive and Gram-negative bacteria), actinomycete and total PLFAs were all significantly increased in the CC3 treatment, and decreased in the CC6 and CC10, and reached to the lowest in the CC15 treatment. However, the PLFA of fungi was increased and reached to the highest in the CC15, which was 182% higher than the control treatment (CK). These results demonstrated that continuous peanut monocropping significantly affected the soil microbial community structure, especially the soil fungal community.

The ordination biplot of CCA displays the effect of continuous peanut monocropping explaining the variance in microbial community composition (Figure 2). The first and second canonical axes explained 86.0% of the variability in the microbial communities. With increasing continuous monocropping years, soil microbial composition changed greatly. In the control treatment (CK), the soil composition changed with relation to some bacteria (i15:0; cy17:0; cy 19:0), while the microbial community was influenced greatly by other bacteria (i15:0; a15:0; i16:0; i17:0; 16:1ω9; 18:1ω7) and actinomycetes in the peanut monocropping for 3 and 6 years treatments (CC3 and CC6). The difference between the CC3 and CC6 was that CC6 was also slightly correlated with some fungi, but CC3 exhibit obvious negative correlation with fungal composition. When the continuous monocropping time exceed 10 years (CC10 and CC15), the soil microbial community was positively correlated with fungal composition and negatively correlated with most bacteria and actinomycetes.

Soil microbial community studied by DGGE

The DGGE profiles of 16S rDNA demonstrated the apparent changes of the bacterial community in different treatments (Figure 3). The bacterial bands increased in the CC3 (41 bands) and CC6 (56 bands) treatments, and decreased in the CC10 (38 bands) and CC15 (36 bands). The dendrograms of soil bacterial diversity (Figure 3B) showed that treatments CC10 and CC15 comprised group one, with a similarity of 88%. Compared with CC6, treatment CC3 was more similar with group one with a similarity of 85%; another group comprised only CK, with a similarity to other groups of less than 55%. This result indicated that the diversity of the bacterial community was altered by continuous peanut monocropping.

The DGGE profiles of 18S rDNA demonstrated the changes of the fungal community in different treatments (Figure 4). The fungal bands decreased in the CC3 (28

Table 4. Effect of continuous peanut monocropping on PLFAs of soil microbial community.

	GP (nmolg^{-1} dry soil)	GN (nmolg^{-1} dry soil)	Bacteria (nmolg^{-1} dry soil)	Fungi (nmolg^{-1} dry soil)	Actinomycete (nmolg^{-1} dry soil)	Total PLFAs (nmolg^{-1} dry soil)	F/B
CK	4.11±1.16c	3.76±0.52c	7.87±0.64d	2.15±0.03c	2.68±0.01b	12.70±0.62c	0.27±0.03c
CC3	9.29±1.70a	8.94±1.77a	18.23±3.47a	4.58±0.11a	4.23+0.54a	27.03±4.12a	0.25±0.04c
CC6	8.10±0.64ab	6.78±1.15ab	14.88±1.79ab	5.17±0.33ab	4.04±0.16a	24.08±2.28ab	0.35±0.02bc
CC10	7.63±0.07ab	5.4±0.01bc	13.03±0.08bc	5.27±0.69ab	3.05±0.31b	21.35±0.91ab	0.40±0.06b
CC15	5.60±0.47bc	4.20±0.35c	9.79±0.13cd	6.06±0.17b	2.62±0.11b	18.46±0.19bc	0.62±0.01a

Values followed by the different letters are significantly different within columns (P<0.05).
GP and GN stand for Gram-positive and Gram-negative bacteria respectively, and F/B is ration of fungi to bacteria.

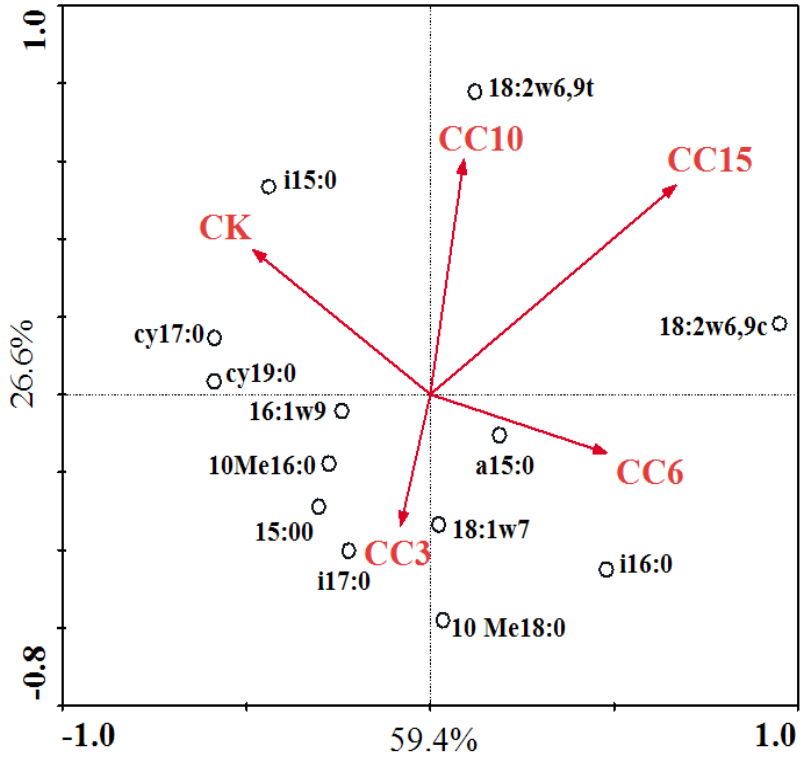

Figure 2. Soil microbial community structure as affected by different years of continuous peanut monocropping indicated by CCA of all PLFAs.

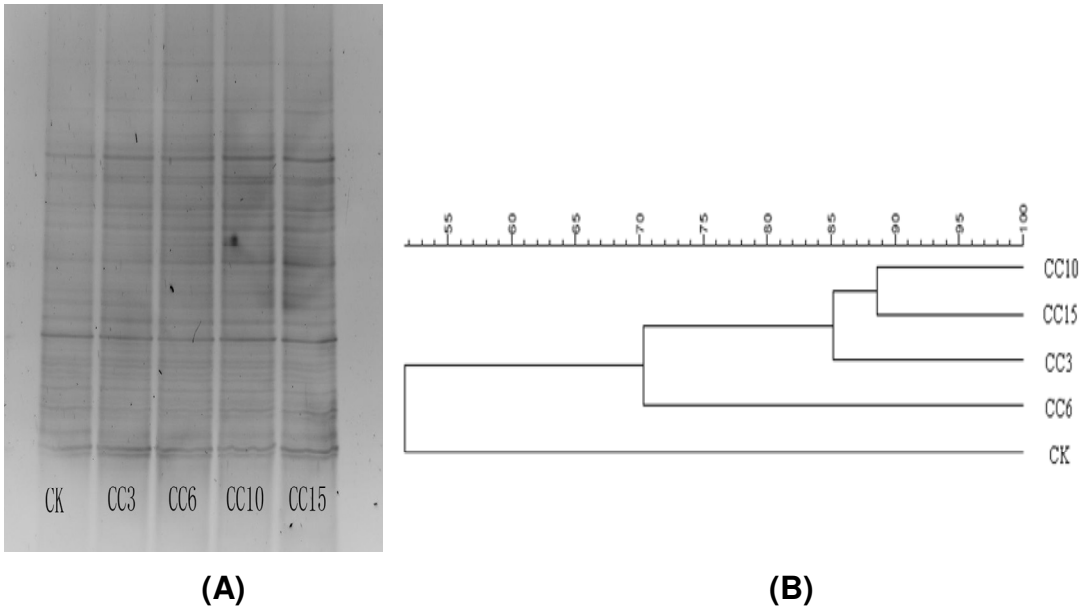

Figure 3. (A) Community structure of bacteria as affected by different years of continuous peanut monocropping indicated by DGGE. (B) Cluster analysis of bacterial 16S rDNA Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis profiles.

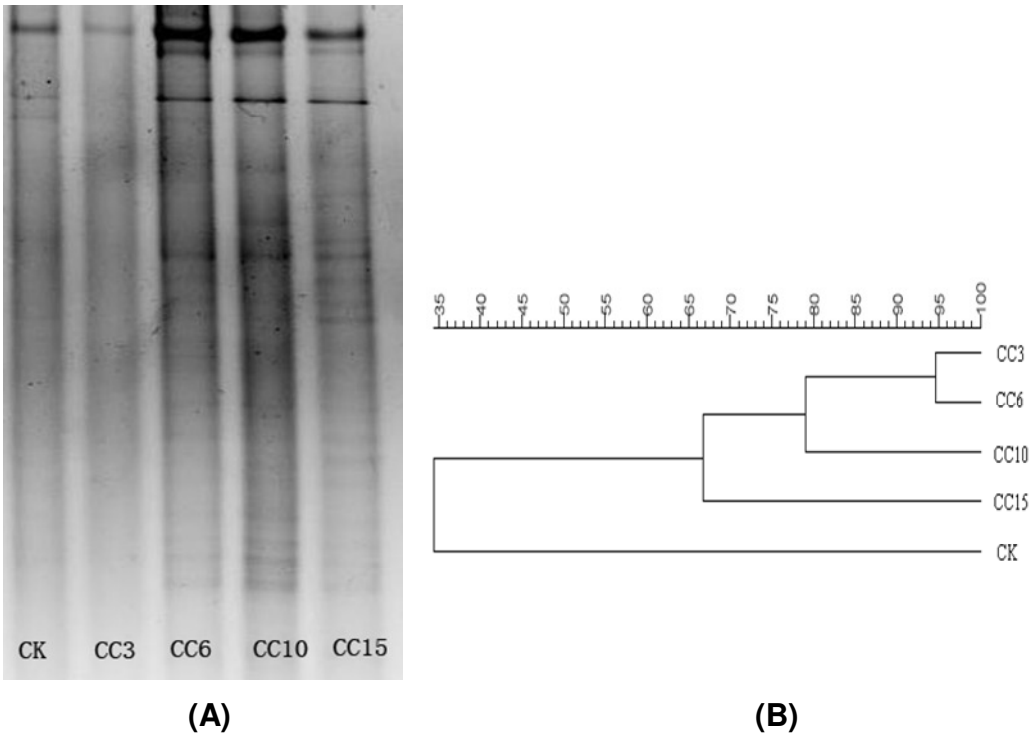

Figure 4. (A) Community structure of fungi as affected by different years of continuous peanut monocropping indicated by DGGE (B) Cluster analysis of bacterial 18S rDNA Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis profiles

bands) and CC6 (24 bands) treatments, and increased in the CC10 (31 bands) and CC15 (36 bands). The dendrograms of soil fungal diversity (Figure 4B) showed that the treatments CC3 and CC6 comprised a group. With

the increase of continuous cropping years, the differences of fungal diversity was more significant. Continuous cropping 15 years, the similarity of fungal communities was 67%, much less than that of CC10. The other group comprised only CK, with a similarity to other groups of less than 35%. This result indicated that the diversity of the fungal community was altered to a greater extent than the bacterial by continuous peanut monocropping.

DISSCUSION

With monocropping years increasing, peanut yield decreased (Figure 1), soil pH also decreased (Table 2) and content of phenolic acids increased in the soils (Li et al., 2010). Lian et al. (2010) also reported that peanut planting decreased significantly pH of red soil. Due to the annual fertilization, total nitrogen, available phosphorus and available potassium in the soil increased after peanut cropped (Table 1), however it was not correlated to the time of monocropping. According to the standard of red soil quality suggested by Soil Quality of China (Cao and Zhou, 2008), the content of soil organic matter is relatively low, but contents of total nitrogen, total P, alkali-hydrolyzable N, available P and available K were relatively high in the soils, it is unlikely that these nutrients are the key limiting factors for peanut growth in the sampling sites. Moreover, peanut yield (Figure 1) was independent of contents of organic matter, N, P and K in the soils (Table 1). However, soil acidification probably decreased peanut pod yield.

Using traditional culture method, continuous cropping has been found to increase the amount of fungi, decrease the amount of bacteria and actinomycetes (Bridge and Spooner, 2001; Chen et al., 1999; Li et al., 2005; Xu and Wang, 2003). In a recent study, Nayyar et al. (2009) concluded that continuous monocropping reduced the number of beneficial Gram-positive bacteria and AM fungi, repressed soil microbial biomass, and induced the accumulation of root pathogens. Yao et al. (2006) also reported that continuous cucumber cultivation caused the reduction in the species diversity of the biota. Our results showed that the population of all kinds of microbes increased within peanut monocropping 3 years by culture dependent method and PLFA. When peanut monocropped over 3 years, the population of bacteria and actinomycetes decreased, and fungal population still increased. CCA analysis also showed that with increasing peanut monocropping years, soil microbial structure become more correlative with fungi compositions. It is noted that, the proportion of bacteria in total PLFA decreased from 67.4 to 53.0%, meanwhile the proportion of fungi increased from 16.9 to 32.8% (Table 4). All these results provides evidence that with increasing mono-cropping years the dominant advantage of soil bacteria was gradually whittled by the increase of fungal population. According to the isolated bands from DGGE profile, bacteria and fungi diversity rose firstly and then decreased with the increasing monocropping years. Moreover, the changes of fungi diversity were much greater than those of bacteria. This was in consistent with the results of He et al. (2008). They found soil fungi seem to be a more sensitive indicator of soil fertility than soil bacteria.

Soil enzyme activities have been suggested as proper indicators of soil quality and functional microbial diversity because they control key metabolic pathways in soils. They could even provide insights into the uptake of nutrients in soils as affected by land management, such as cropping systems (Insam, 2001; Klose and Tabatabai, 2000). Invertase and urease were the most prominent enzymes in the cycling of carbon and nitrogen in soils, and polyphenol oxidase was one of the important redox enzymes in the transformation of benzene-containing compounds. In this study, the invertase and urease activities in the continuous cropping system decreased with the increasing cropping years. This result was consistent with the precious study in Fluvo-aquic soil (Sun et al., 2001). Polyphenol oxidase activity increased at first several years and then decreased, implying that the decomposition of benzene compound might have been hindered. The reduction of polyphenol oxidase activity could lead to the accumulation of phenolic acids in soil, potentially causing peanut autotoxicity. Generally, soil bacteria was the dominant microbes to proceed soil nutrients cycle. In our study, the decrease of invertase and urease activities was probably correlated with the decrease of soil bacterial population and diversity.

Soil microorganisms can greatly influence on soil and plant processes and functions. They are critical factors of soil nutrient status, crop health and overall crop productivity. An abundance of beneficial soil organisms can suppress pathogens and diseases, improve nutrient availability, promote plant growth, and thus increases yield (Larkin, 2003). Acosta-Matinze et al. (2008) reported that there was higher soil microbial biomass, some of the fungal and bacterial fatty acid methyl esters (FAMEs) in continuous peanut monocropping system, compared with peanut-cotton rotation systems after 8 years. However, there are lower yield and higher costs due to higher disease and nematodes in continuous peanut mono-cropping system (Lemon et al., 2001). Although the observed increased in fungal communities with peanut monocropping years in our study can be related to potential increase in soil C sequestration because they have higher C assimilation than bacteria (Bailey et al., 2002), the invertase and urease activities showed a decreased with monocropping years, the fungal species or phyla increased over years of monocropping in these sites may represent a high number of pathogens or species that have no significant role in the synthesis of enzymes in the soil in this study, since diseases such as root rot, bacterial wilt, souther blight, increased with

monocropping peanut years in the red soil region in subtropical China (Wang and Chen, 2005) .

Our previous research showed *p*-hydroxybenzoic acid, vanillic acid and coumalic acid accumulated gradually with the increasing year of continuous peanut monocropping, and confirmed their allelopathy in inhibiting peanut growth, increasing the incidence of peanut infected with pathogen and decreasing the incidence of peanut seed germination infecting with pathogen (Li et al., 2010). In this study, the initial increase and latter decrease of polyphenol oxidase activity during 3-10 years probably attribute to the dynamic of microbial community. While the increase of soil polyphenol oxidase activity in the peanut monocropping for 15 years treatment may due to the long-term accumulation of peanut root exudates such as phenolic acids and the tendency of fungal community.

In summary, with the increasing years of continuous peanut monocropping, the peanut yield decreased. The contents of organic matter, N, P and K in the soils were hardly affected by peanut monocropping years, while soil pH decreased with time. Both culture dependent and molecular method showed that continuous monocropping could greatly change the soil microbial structure. The increasing populations of soil fungi weaken the dominant position of bacteria in the soils. The fungal CFUs and species increased over years of monocropping in these sites. Soil acidification and the changes in soil microbial community also cause the changes in soil functional enzymes activities. This may slow down the soil carbon and nitrogen cycle, and induce the accumulation of crops autointoxicants.

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