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REGULAR ARTICLE

Changes in the abundance and structure of bacterial communities under long-term fertilization treatments in a peanut monocropping system

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Abstract
Background and aims Peanut yield and quality are seriously compromised by continuous monoculturing in the red soil region of southern China. Monoculturing can cause soil degradation and an increase in soil-borne diseases. This research aimed to investigate the influence of long-term peanut monocropping and different fertilization treatments on peanut growth, soil physical and chemical properties and soil microbial community.
Methods A long-term fertilization experiment established in 1996 was utilized to examine the effect of various fertilization treatments including chemical and organic fertilizers treatments. Deep 16S rRNA gene pyrosequencing highlighted changes in the abundance and structure of bacterial communities, especially of the pathogenic and beneficial bacterial communities in long term chemical fertilizer treatment in comparison to the organic manure treatment.
Results Chemical fertilizer treatment causes a shift in bacterial community structure and decrease in diversity under the long-term monocropping in comparison to organic fertilizer. The abundance of the bacterial pathogen *Ralstonia solanacearum*, a causative agent of peanut wilt, was found to be associated with a loss of community diversity and loss of the peanut yield.
Conclusions The organic fertilizers more effectively increase microbial diversity in the soil and changed the community structure. Long-term use of the chemical fertilizer leads to a decrease in microbial diversity of the soil and an increase in *R. solanacearum* with associated increase of peanut wilt. The potential decrease in diversity and competition between the bacterial community and the pathogen may be a contributing factor to increased disease during long-term chemical fertilizer use.

Keywords Peanut monocropping · Chemical fertilizer · Organic fertilizer · Bacterial community · *Ralstonia solanacearum*

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Introduction

Peanut (*Arachis hypogaea* L.) is the predominant upland crop in the hilly red soil region of southern China (Wang and Chen 2005). The warm climate, sunlight and

moisture are adequate for production but production is often hindered due to poor fertility and strong acidity of the red soil (Liu and He 1991). Peanut plants have generally been monocropped continuously on a large scale in these regions due to limited arable land and a requirement for intensifying regional agro-industrialization (Lian et al. 2010). Numerous studies have indicated that consecutive monoculturing can lead to crop yield and quality decline and increased disease pressure (Larkin 2003; Li et al. 2012). 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.

Soil microorganisms are vital to agroecosystem function and sustainability but are sensitive to changes in land management practices, such as cropping system, tillage and fertilization (Zhou et al. 2014). Consequently, soil microbial parameters such as microbial composition and diversity have been suggested as possible indicators of soil quality and soil function (Kong et al. 2011). It is reported that crop rotation and organic matter inputs can increase the soil microbial biomass enhancing soil enzymatic activities involved in nutrient transformation which leads to improved soil quality and enhanced soil function (Acosta-Martínez et al. 2010). Reports also indicate that chemical fertilizers which introduce N (nitrogen), P (phosphorus), and K (potassium) can also lead to the increased soil productivity and improve soil microbial properties such as microbial biomass C and N, and microbial diversity (McAndrew and Malhi 1992; Wu et al. 2011). Improvements to crop yield and soil carbon over the short-term has been reported in some studies investigating the influence of chemical and organic fertilizers applications on soil bacterial diversity and community function (Crecchio et al. 2001; Peacock et al. 2001). In contrast, other studies have also found that most of the microbiological characteristics studied did not differentiate between fertilized and non-fertilized treatments over a longer time period of up to many years (Sarathchandra et al. 2001; Freitag et al. 2005). Studies reporting the microbial communities changes in response to fertilization treatments have mostly been performed by traditional molecular technique such as Denaturing Gradient Gel Electrophoresis (DGGE) (Ribeiro et al. 2013). These studies are often limited due to low resolution taxonomic information provided by DGGE and by limits in the ability of DGGE to detect low abundance bacteria groups present in the soil. This may cause important small-scale community members to be over looked (Chen et al. 2013). Therefore, more advanced techniques are needed to detect key changes in the microbial ecology within the low abundance bacteria groups. In recent years, the next generation pyrosequencing technology has allowed 16S rRNA gene sequencing, at a high throughput and low cost, more indepth microbial community diversity investigations (Das and Kazy 2014).

Soil-borne diseases have become a prevalent problem in the production of many annual crops subject to intensive monocropping (Li et al. 2014a). To date, many studies concentrated on fungi as a causative agent of for the crop productivity decline. Li et al. (2014b) reported that the fungal pathogens, such as *Leptosphaerulina* sp., *Fusarium* sp., were the dominant fungal pathogens responsible for peanut disease and yield declines over the consecutive peanut monocultures. However, peanut sickness and decline caused by bacterial pathogens has not been studied in this red soil region. Our previous study showed peanut bacterial wilt caused by *Ralstonia solanacearum* tended to increase in prevalence with increasing monocropping years (Wang et al. 2011). Peanut wilt is a soil-borne bacterial pathogen notorious for its lethality, persistence, complex subspecies, wide host range, and broad geographic distribution (Allen et al. 2005). Nevertheless, the bacterial community structure and diversity shifts under the different long-term fertilization were rarely studied by the method of pyrosequencing and an interaction with *R. solanacearum* has not been detected.

This research aimed to investigate the influence of long-term peanut monocropping and different application of fertilizer on peanut growth, soil physical and chemical properties and soil microbial community. To achieve this aim agricultural soil samples from the red soil region of southern China that had been subject to either: 2 years of peanut monocropping using chemical fertilizer (S-CF) (Previously planted with kiwi fruit prior to peanut monocropping treatment); long-term (16 years) of continuous monocropping with chemical fertilizer (L-CF) or; long-term (16 years) of continuous monocropping with organic manure amendments (L-OM). Soil analysis and deep sequencing revealed the relationship of different fertilization regimes with pathogens incidence, offering useful information for

bacterial wilt disease suppressive and better guidance to peanut cropping.

Material and methods

Description of the experimental site and sampling

The long-term field fertilizer experiment of peanut (Ganhua 5) continuous monocropping was initiated in 1996 at Ecological Experimental Station of Red Soil, Chinese Academy of Sciences, Yingtan, southern Jiangxi Province of China (116°55′E, 28°12′N). The region has a typical subtropical monsoon climate with an annual precipitation of 1795 mm, annual evaporation of 1318 mm and a mean annual temperature of 17.6 °C, with an average of 262 frost-free days. The tested soil was classified as a Haplic Stangnic Anthrosol and derived from quaternary red clay. Soil samples (0–20 cm) taken from experimental site in April 1996 at the beginning of the experiment contained 0.6 g kg^{-1} total N, 0.2 g kg^{-1} total P, 14.5 g kg^{-1} total K, 43.3 mg kg^{-1} hydrolysable N, undetectable available P ($NaHCO_3$-extractable P) and 84 mg kg^{-1} available K (NH_4OAc-extractable K), 8.4 g kg^{-1} organic matter (OM) and had a pH of 3.90 (Table 1). The 2 years of peanut monocropping with chemical fertilizers (S-CF) was also conducted at the same site and initiated in 2010.

Fertilization treatments

Peanut (Ganhua 5) was sowed manually on April 10th of each year by placing two seeds per hole to give a 10 cm plant-to-plant spacing and 30 cm row-to-row spacing. The plot site was used for either 16 years of continuous peanut monocropping or 2 years of peanut monocropping. Plots received one of 3 treatments: (L-CF) long-term (16 years) of peanut monocropping with chemical fertilizer of N, P, K at 45, 45, and 135 kg ha^{-1}, respectively; (L-OM) long-term (16 years) of peanut monocropping with organic fertilizer (pig manure); and (S-CF) 2 years of peanut monocropping with chemical fertilizer of N, P, K at the same rate as the L-CF treatment. The treatments were arranged in a randomized complete block design with four replicates. Each plot was 5.5×6.0 m with a concrete wall embedded 20 cm into soil between each plot. N, P and K were supplied as urea, calcium magnesium phosphate and potassium chloride in treatment L-CF and S-CF, respectively. Pig manure in treatment L-OM was bought after fermenting from a nearby pig farm every year and 1 kg air-dried pig manure sample was collected at the time of application to determine its components. Pig manure was added at the rate of 1.69 t ha^{-1} (dry weight). The average composition of the pig manure was pH 8.5, OM 602, N, P, K 26.7, 18.3 and 53.1 g kg^{-1}, respectively. 14 kg P ha^{-1} of calcium magnesium phosphate and 45 kg K ha^{-1} of potassium chloride was added to the L-OM treatment to attach the equal final rates of total N, P, K to the L-CF treatment (45, 45, and 135 kg ha^{-1}, respectively). All of fertilizer was applied to the surface of the soil as base fertilizer 1 day before sowing peanut.

Soil characteristics analysis and quality analysis of peanut kernel

Soil sampling was conducted in sowing season (April), flowering season (June), and harvest season (August) in 2012. Five soil cores (0–20 cm) of bulk soil were taken randomly across each plot from the root zone in each replication. The soil was then mixed and homogenized by passing through<2 mm sieve to remove aboveground plant materials, roots, and stones and stored at −70 °C prior to soil DNA extraction and at 4 °C for other analyses. Selected characteristics of the soil samples are listed in Table 1. Soil samples taken from the experimental area in 2012 were analyzed. Chemical properties of: pH; OM; total N, P, K; hydrolysable N; and available P, K in the soil samples were determined using routine methods (Lu 1999).

Peanut plants were harvested manually in August and all harvested biomass was removed from the plots. Yields were determined by removing peanut plants, air-drying in the field for 4–6 d on concrete, threshing and oven drying at 65 °C to a constant moisture level, and then weighing.

Soil DNA extraction and PCR

DNA was isolated from 0.5 g of mixed soil using the FastDNA Kit (Qbiogene Inc., CA, USA) and purified on agarose gels (Moreira 1998). The primer pair 515f and 907r (515F 5′- GTGCCAGCMGCCGCGG-3′, 907R 5′- CCGTCAATTCMTTTRAGTTT-3′) were utilized to amplify a 392 base pair fragment of the 16S rRNA gene for 454 pyrosequencing (Xu et al. 2013).

An aliquot of 1uL DNA was used in the PCR reaction. The PCR conditions used for amplification were 94 °C for 2 min, 20 cycles of 94 °C, 45 s denaturation; 55 °C, 45 s annealing and 72 °C, 1 min extension;

Table 1 Soil properties under different fertilization management and sampling time

Treatments[a)]	pH	Organic Matter (OM) g kg^{-1}	Total N g kg^{-1}	Total P g kg^{-1}	Total K g kg^{-1}	Hydrolysable N mg kg^{-1}	Available P mg kg^{-1}	Available K mg kg^{-1}
Initial soil (1996)	3.90±0.03 d	8.4±0.6 d	0.60±0.08 d	0.20±0.01 g	14.5±0.4 a	43.3±3.9 e	–	84±7 e
1S-CF	4.10±0.04 d	6.9±0.8 d	0.47±0.09 e	0.25±0.04 f	10.7±0.6 e	44.2±6.7 e	4.1±0.4 d	83±5 e
1L-CF	5.14±0.15 bc	13.5±0.5 c	0.80±0.03 c	0.37±0.02 de	12.2±0.4 cd	56.9±8.6 cd	5.6±0.2 d	292±25 d
1L-OM	6.41±0.21 a	15.2±0.3 b	0.96±0.05 b	0.97±0.06 ab	13.7±0.1 b	60.4±0.4 bcd	126.4±5.6 a	454±48 c
2S-CF	4.08±0.06 d	7.1±0.8 d	0.47±0.02 e	0.30±0.06 ef	11.8±0.1 cd	53.5±1.3 d	4.9±0.9 d	98±13 e
2L-CF	4.97±0.17 c	12.7±1.4 c	0.82±0.03 c	0.39±0.02 cd	12.6±0.4 c	60.5±6.8 bcd	7.0±0.7 d	346±34 d
2L-OM	6.46±0.06 a	15.9±0.8 b	0.97±0.02 b	1.01±0.06 a	14.7±0.1 a	63.5±1.2 abc	97.9±9.0 b	529±50 a
3S-CF	4.13±0.11 d	8.1±1.2 d	0.58±0.04 d	0.42±0.03 cd	10.7±0.9 e	58.9±3.7 cd	4.4±0.3 d	114±14 e
3L-CF	5.28±0.14 b	15.6±0.8 b	0.91±0.04 b	0.45±0.04 c	10.9±0.5 e	66.9±5.2 ab	4.9±0.8 d	514±51 bc
3L-OM	6.63±0.11 a	18.1±1.2 a	1.08±0.04 a	0.92±0.03 b	11.7±0.9 d	71.9±3.7 a	59.0±1.3 c	644±54 a

[a)] S-CF, 2 years of peanut monocropping with chemical fertilizer; L-CF, long-term (16 years) of peanut continuous monocropping with chemical fertilizer; L-OM, long-term (16 years) of peanut continuous monocropping with organic fertilizer. The numbers 1, 2, 3 represent different sampling times (April, June and August) in 2012, respectively

Statistical significance was set at a level of $p<0.05$ using Duncan's multiple range tests. The same letter in table represents no significant difference. Values are means±standard errors ($n=4$)

followed by 72 °C, 6 min. After 20 rounds of amplification, a further three rounds of amplification added the A and B adapters to specific ends of the amplified 16S rRNA fragment (Margulies et al. 2005). Sequencing was conducted by Majorbio Bio-pharm Technology Co., Ltd (Shanghai, China).

Processing of pyrosequencing data

The 16S rRNA gene sequence data was analyzed by the pyrosequencing pipeline tools available from the Ribosomal Database Project (RDP) and MOTHUR version 1.24.1 (Schloss et al. 2011). The counts of the number of the sequences of each cluster within each sample were converted to frequencies by dividing the number of counts of each cluster by total number of sequences generated within each sample. The 16S rRNA sequences were first trimmed and then sequences with<200 bases were removed from the data sets with MOTHUR. The total number of sequences in each library ranged from 6978±602 to 9226±1232 among the treatments. Sequences were submitted to the RDP aligner tool for species identification. Further processing analyses was then carried out using the MOTHUR v.1.24.1. The resulting clusters were assessed at 97 % dissimilarity to provide the data needed for diversity analysis. Richness estimators (Chao and Ace) for sample size of 6000 sequence and diversity indices (Shannon and Simpson) were calculated using the MOTHUR program at the cutoff of 97 % (Colwell 2009).

Statistics and data analysis

Statistical analysis was carried out with the software package SPSS 19.0 software. Duncan's multiple range tests were used to compare the means of treatments. Variability in the data was expressed as the standard errors. Differences at $p<0.05$ were considered statistically significant. Bacterial community structure based on all sequences was performed by principal coordinates analysis (PCoA) conducted in R (Version 3.1.2) was used to depict differences in composition and structure of the bacterial communities among the three treatments. Canonical corresponding analysis (CCA), performed on the sequences of 16S r RNA of the likely five associated pathogenic and beneficial bacterial, was used to analyze the relationship between the related

associated pathogenic and beneficial bacterial sequence patterns and treatments with environmental variables in three treatments at the harvest time (August).

Results

Effect of consecutive cropping and fertilization on peanut yield, height and soil properties

Peanut kernel yield and height in upland red soil from 1996 to 2012 are presented in Fig. 1. The continuation of continuous cropping with organic matter (L-OM) consistently produced more peanut kernel yield and plant height than in L-CF but both showed yield and height declines over 16 years. The peanut yield and height in L-OM treatment group decreased from 3424 kg hm^{-2} and 54.0 cm in 1996 to 678 kg hm^{-2} and 36.3 cm in 2012 while L-CF decreased from 2876 kg hm^{-2} and 36.0 cm in 1996 to 307 kg hm^{-2} and 29.6 cm in 2012. The peanut height increased slightly in 1999 by the influence of climate, but presented a downward trend overall the long-term fertilization experiment. The S-CF treatment produced a peanut kernel and height of 2218 kg hm^{-2} and 41.8 cm in 2012. Soil properties (Table 1) indicated that the soil pH in L-CF (5.14) and L-OM (6.41) treatments were higher than S-CF treatment (4.10) at the sowing time in 2012. While there was a trend in an increase of pH in all soil samples from the original soil sampled in 1996 (pH 3.90), none of the treatments produced a significant difference between sowing and harvest within a single year. The partial neutralization of soil acidity in L-OM and L-CF treatments compared to the S-CF is likely due to the pig manure or calcium magnesium phosphate in the chemical fertilizer application over a long period (1996–2012). Continuous cropping over a 16 years period had seen an increase in organic matter from 8.4 g kg^{-1} in the original 1996 samples at the start of the long term monoculturing to 13.5 g kg^{-1} (L-CF) and 15.2 g kg^{-1} (L-OM) in the final year (2012) (Table 1) Additionally, soil N, P and K contents in all treatments significantly increased after 16 years of cropping and fertilization. Moreover, soil OM, N, P and K were all higher in pig manure treatments than in chemical fertilizer treatments, showing that application of organic manure played a more important role in enhancing soil fertility.

16S rRNA pyrosequencing

The 16S rRNA gene survey produced a total of 182,870 sequence reads with a length between 250~550 bp from 36 samples. An average of 5946±480, 4675±548 and 5619±750 (mean±SD) bacterial sequences were obtained from the soil samples of the S-CF, L-CF and L-OM treatments at the sowing time, respectively. The structure of the microbial communities in the soil samples from the three peanut treatments at the three sampling times were compared at the level of phylum level (Fig. 2). Overall, about 80–90 % of all reads obtained could be assigned to phylotypes, with approximately 10–20 % of the reads in each sample remaining unclassified. Both species richness and diversity indices of the

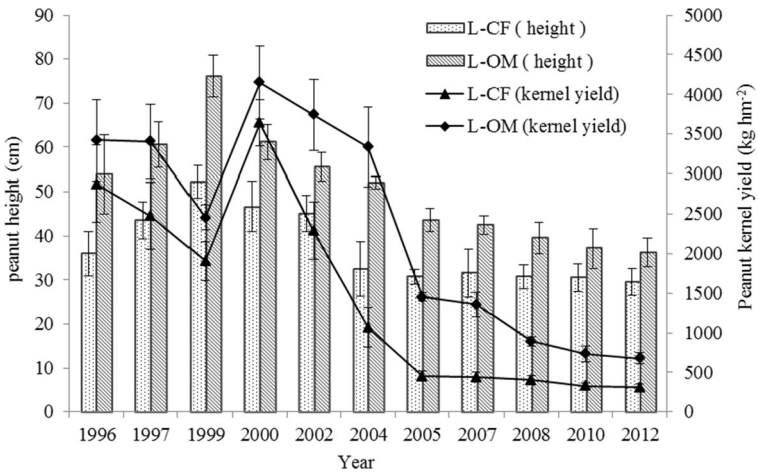

Fig. 1 Effect of long-term chemical or organic fertilizers on peanut height and kernel yield in upland red soil from 1996 to 2012. *Error bars* indicate standard deviation (SD) (n=4)

S-CF treatment and L-CF treatment increased in flowering time (June) and then decreased in harvest time (August), while kept rising with the peanut growth in the L-OM treatment despite of no significant difference being observed (Table 2). The order of the ACE (species richness) index changed from sowing time (S-CF > L-OM > L-CF) to harvest (L-OM > S-CF > L-CF). Other richness estimators (Chao) and diversity indices (Shannon and Simpson) corroborate this trend (Table 2).

Effect of different long-term fertilizer management of peanut on bacterial community composition

The structures of the overall bacterial communities in the soil samples from the three peanut treatments at the three sampling times were compared at the phylum level (Fig. 2). The results revealed that long-term fertilization regimes resulted in the changes of soil bacterial community structure in the red soil. The relative abundance of *Actinobacteria* increased from sowing to flowering for L-CF and L-OM treatments, and then declined at harvest, yet *Bacteroidetes* and *Nitrospirae* increased across all the growth periods. The bacterial community composition of the organic manure treatment was consistently different to the two chemical fertilizer treatments particularly for the beneficial phylum *Nitrospira* that was more abundant in the organic matter treatment. *Acidobacteria* was found to be negatively correct with pH value (Mannisto et al. 2007; Lauber et al. 2008; Fierer 2009). Here, perhaps due to the higher pH, the relative abundance of *Acidobacteria* was always lower in the L-OM treatment compare with other treatments. In August, the relative abundance of soil-borne pathogen *Ralstonia* sp. achieved 0.16, 0.48, and 0.28 %, compared to 0.04, 0.03, and 0.01 % in April, in the S-CF, L-CF and L-OM treatments, respectively (Fig. 4). *Ralstonia* sp. was always higher in the L-CF treatment than in the L-OM treatment (Fig. 3). The field investigation and observation on incidence rate of peanut bacterial wilt caused by *Ralstonia* sp. also showed that the ratio of peanut wilt is only 8 % in S-CF treatment, while the ratio of peanut wilt were 58 and 22 % in L-CF and L-OM treatments, respectively. At the same time, the abundances of some beneficial bacterial such as *Streptomyces* sp., *Sphingomonas* sp., *Bacillus* sp. *and Arthrobacter* sp. increased significantly in the three treatments at the different sampling times (Fig. 4).

Principal coordinates analysis clearly showed that the first principal component differentiated the two long-term treatments with L-OM on the positive side of the x-axis and L-CF on the negative side, suggesting large differences in the

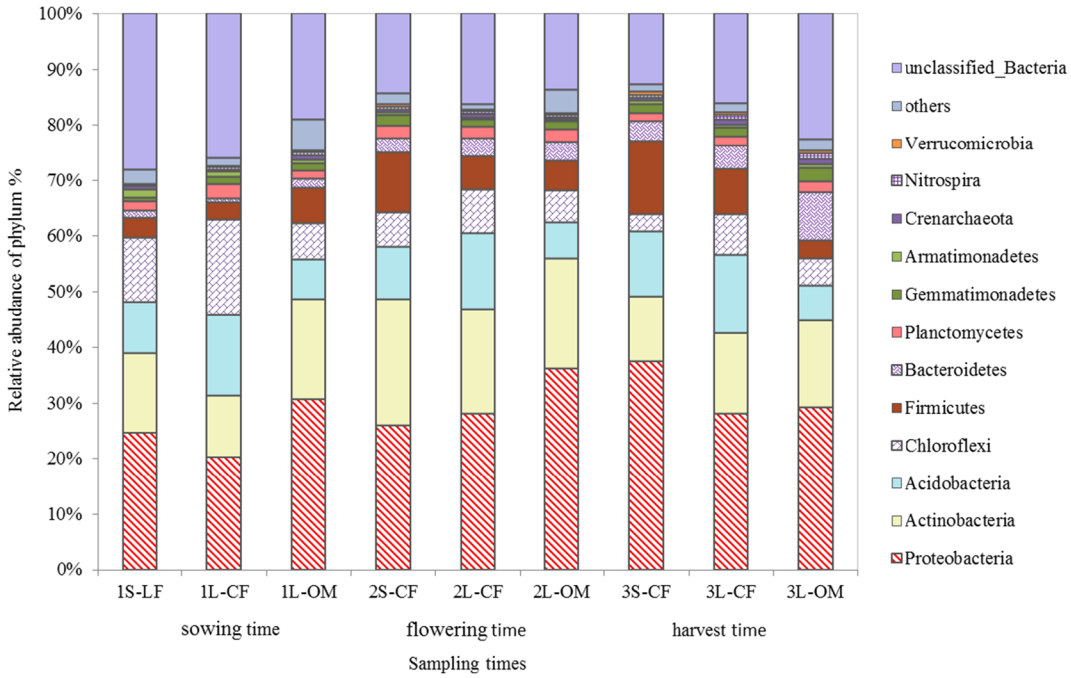

Fig. 2 The frequencies of sequences over phylum level as affected in the soil of the treatments S-CF, L-CF, and L-OM at three stages of peanut development. All of the samples were collected in 2012. The numbers 1, 2, 3 represent as above

Table 2 Richness estimators and diversity indices [a)] of soil bacterial community of the experiment groups with the process of treatment

Treatments	Richness estimators		Diversity indices	
	ACE	Chao	Shannon	Simpson
1S-CF	5946±480 c	4161±396 cd	6.54±0.46 bcd	0.0068±0.0002 de
1L-CF	4675±548 d	3461±335 d	6.23±0.27 d	0.0051±0.0004 e
1L-OM	5619±750 c	4148±336 cd	6.48±0.25 cd	0.0062±0.0002 d
2S-CF	7956±447 ab	5159±478 ab	6.93±0.23 abcw	0.0088±0.0005 c
2L-CF	7279±551 b	4331±761 bcd	6.99±0.24 abc	0.0036±0.0001 f
2L-OM	8046±648 ab	5142±733 abc	6.90±0.45 abc	0.0170±0.0017 a
3S-CF	7581±273 b	5097±213 abc	7.05±0.14 ab	0.0090±0.0007 c
3L-CF	6097±396 c	4326±160 bcd	6.84±0.13 abc	0.0038±0.0003 f
3L-OM	8800±688 a	5763±796 a	7.21±0.24 a	0.0121±0.0001 b

[a)] The richness estimators and diversity indices were calculated based on equal number of sequences (6000 sequences). The numbers 1, 2, 3 represent as above

Statistical significance was set at a level of $p<0.05$ using Duncan's multiple range tests. The same letter in table represents no significant difference. Values are means±standard errors ($n=4$)

bacterial community composition of these two treatments (56.6 % variation on the x-axis). The two chemical fertilization treatments (L-CF and S-CF) cluster separately, though both groups are on the negative side of the x-axis. It indicated that organic matter fertilization was a dominant factor influencing the bacterial community composition. The second principal component axis accounted for 20.2 % of the variation amongst samples and clear separation can be seen on this axis between the S-CF and the L-CF and L-OM. This separation most likely reflects that long-term fertilization ameliorated the soil physicochemical properties, thus lead to greater impact on the bacterial community than that in the short time S-CF (Fig. 5). Similar clusters were found on the different treatments at the sowing (Fig. S1) and flowering season (Fig. S2).

Canonical correspondence analysis (CCA) was used to reveal what environmental factors shifted bacterial assemblages in soils. CCA related the changing pattern of bacterial genus and different treatments to different environmental variables such as pH value, total N (TN), P (TP), and K (TK) contents, and hydrolysable N (HN), available P (AP), available K (AK) contents (Fig. 6). Statistical analysis showed that soil properties observed for L-OM could be distinguished from S-CF and

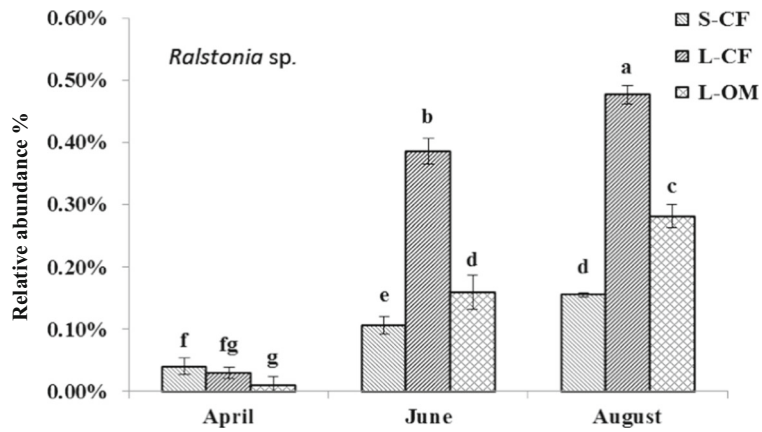

Fig. 3 Relative abundances of *Ralstonia* sp. pathogen at the genus level, in three treatments (S-CF, L-CF, L-OM) at the three stages of peanut development. Error bars indicate standard deviation (SD) ($n=4$). Different letters above bars denote statistical significance at $p<0.05$, according to Duncan's tests

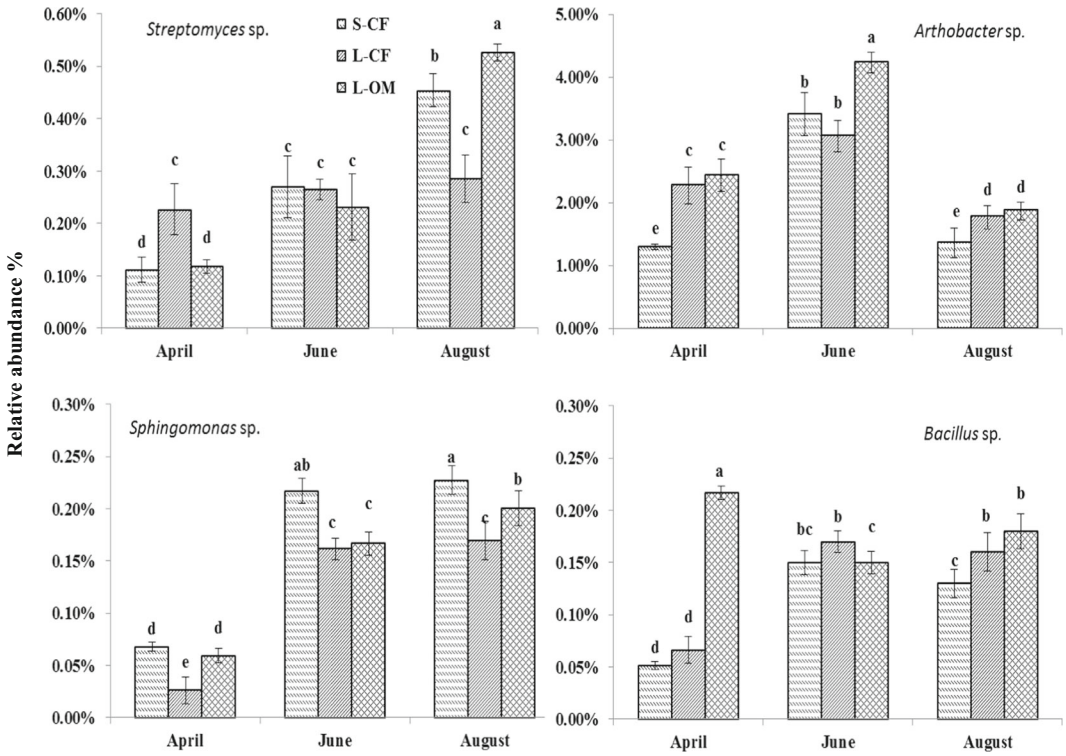

Fig. 4 Relative abundances of four groups of plant beneficial strains at the genus level, *Streptomyces* sp., *Arthobacter* sp., *Sphingomonas* sp., and *Bacillus* sp. in three treatments (S-CF, L-CF, L-OM) at the three stages of peanut development. *Error bars* indicate standard deviation (SD) ($n=4$). Different *letters above bars* denote statistical significance at $p<0.05$, according to Duncan's tests

L-CF treatments, which indicated that long-term organic fertilization was more helpful to increase soil fertility and stimulate the growth. Due to the acidic property of the investigated soil, the long-term fertilizations increased soil pH value. For the associated pathogenic and beneficial bacterial community genus, CCA further revealed that organic fertilization (L-OM) increased numbers of beneficial bacterial, such as *Arthrobacter, Bacillus* and *Sphingomonas* species. In contrast, chemical fertilization (L-CF) increased numbers of pathogenic bacterial, *Ralstonia*.

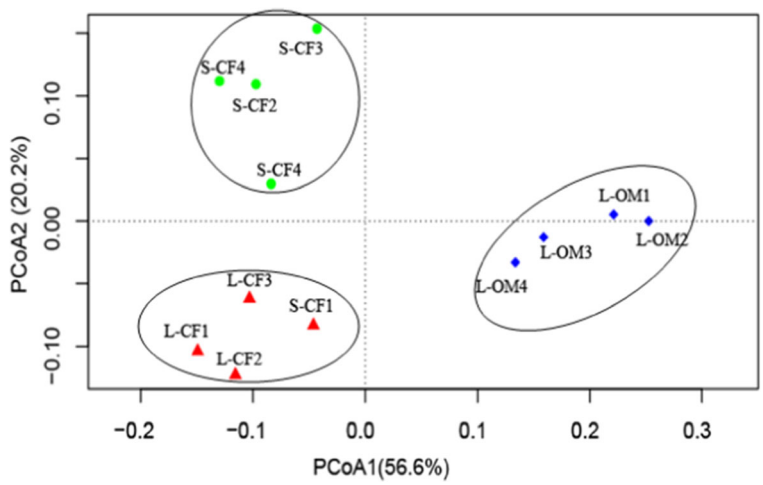

Fig. 5 Comparison of bacterial 16S rRNA communities by different treatments was shown using principle coordination analysis (PCoA) based on the total genera (August). The percentages in *parentheses* indicate the proportions of variation by each ordination axis. The numbers 1, 2, 3, 4 represent four replicates of each treatment, respectively

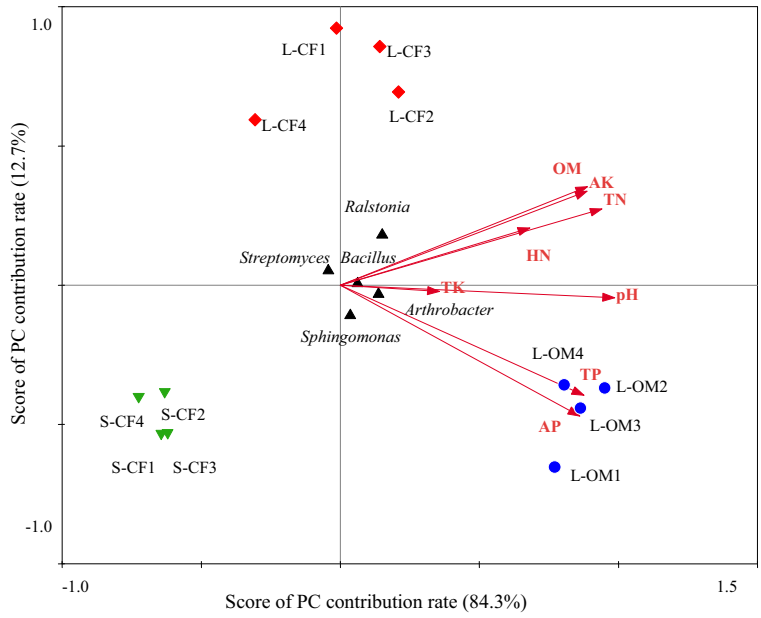

Fig. 6 Canonical correspondence analysis (CCA) relating soils' five associated pathogenic and beneficial bacterial sequence patterns and treatments with environmental variables in 3 different fertilizations treatments at the harvest time (August)

Discussion

Continuous declines in crop yield and quality caused by the continuous monocropping has caught widespread attention (Nishio and Kusano 1973; Liu et al. 2008; Nie et al. 2008). In our study, the peanut yield and height significantly declined when grown in soil that had been continuously cropped for 16 years with annual fertilization from either chemical fertilizers or organic manure (Fig. 1) compared to soil that had received only 2 years of peanut monocropping (S-CF) treatment (data not shown). Observations during our trials found that peanut bacterial wilt, caused by *R. solanacearum*, was higher in long-term cropping treatments compared with the S-CF treatment. The application of organic fertilizers saw an increase in yield and height (Fig. 1) as well as a decrease in the soil-borne disease compared with the chemical fertilizer treatment.

The poor fertility and strong acidity of the red soil derived from quaternary red clay are also important factors that hindered the development of agricultural production (Liu and He 1991). However, due to the long term annual fertilization of the peanut crop, the soil physicochemical properties including soil pH, organic matter, soil total N, P, K and hydrolysable N, available P, available K contents increased significantly in L-CF and L-OM, compared to the S-CF treatment in this study (Table 1). The marked enhancement in the soil organic matter content in both L-OM treatment and L-CM treatment may be due to plant growth and the increased carbon inputs from physical components i.e. shoots and roots. Previous study showed that as much as 40 % of a plant's photosynthate can be deposited in the soil as sugars, organic acids, and larger organic compounds (Kumar et al. 2006). Additionally, apart from N, P, K and other nutrients manure is also rich in organic matter that may improve soil structure, aeration, soil moisture-holding capacity (Reeves 1997; Watts et al. 2010). The total contents of these nutrients were higher in the L-OM treatment than in the L-CF treatment, which indicated the L-OM treatment could better promoting the growth of peanuts as observed in increase plant height and kernel yield. Many studies have indicated that these changes in soil physicochemical properties could also lead to significant microbial differences (Marschner et al. 2003, 2004; Saha et al. 2008). Girvan et al. (2003) reported that soil properties could be a dominant factor controlling the bacterial community composition. Soil pH has also been reported as a predictor of bacterial community structure. Lauber et al. (2009) have demonstrated the overall bacterial community's diversity was correlated with soil pH.

It's reported that the long-term fertilization not only greatly changed the soil physicochemical properties, but also influenced the bacterial community composition and structure (Esperschütz et al. 2007; Melero et al. 2008; Sarathchandra et al. 2001). Soil microbial communities can greatly influence the productivity and overall quality of the agricultural ecosystem due to roles in nutrient cycling, detoxification processes and soil aggregate stability, among other functions (Naeem and Li 1997; Bell et al. 2005; Cardinale et al. 2006; Costa et al. 2007). The long-term fertilization regimes resulted in the changes of soil bacterial community structure and diversity during the growth stage in 2012, and the overall bacterial community structure of L-OM treatment were significantly different from the L-CF treatment (Figs. 2 and 5). A possible benefit to the application of organic fertilizer is an associated increase in the abundance and activity of soil biota, such as soil microorganisms, protozoans, nematodes, collembolans and earthworms (Cao et al. 2011).

The results of this study showed that long-term fertilizations treatments had led to great changes in bacterial community structure. The most dominant bacterial group in this soil was the *Proteobacteria*, similar to a report for several soils, which has great importance to global carbon, nitrogen and sulfur cycling (Spain et al. 2009). *Actinobacteria* was the second most abundant phylum and on average proportion abundance across the treatments was found to be S-CF > L-OM > L-CF (Fig. 2). Previously lower relative abundance of *Actinobacteria* under long-term chemical fertilizer compared with organic fertilizer has been reported (Chaudhry et al. 2012). Similar to previous study, *Acidobacteria*, the third most abundant phylum, did not correlate with nutrient level but did correlate to pH. The abundance of *Acidobacteria* was negatively correlated with pH and decreased as a percentage of sequences in L-OM treatment compared with the other two treatments. Several studies have reported that most known antibiotics from a bacterial origin, such as streptomycin, oxytetracycline, tetracycline, gentamicin, are produced by members of *Actinobacteria*. *Actinobacteria* can survive in the soil environment and are thought to be beneficial in the agricultural soils (Marta et al. 2014). *Acidobacteria* also display a functional capability and specialize on degradation of plant-derived organic matter (Naumoff and Dedysh 2012). Members of *Bacteroidetes*, which were often involved in the degradation of bio-macromolecules such as proteins, cellulose, chitin, pectin, agar, starch (Hugenholtz et al. 1998), and more recalcitrant compounds (Lipson and Schmidt 2004), increased proportionally in the L-OM treatment. *Planctomycetes* were positively correlated with soil microbial biomass C and N, and involved in carbon and nitrogen cycles (Chistoserdova et al. 2004; Justin et al. 2012) but constituted a minor component of the community. Their abundance has previously been reported to be influenced by soil management and compost addition (Buckley et al. 2006). *Nitrospirae* was also present in minor abundance but increased in all treatments during the peanut plant growth phase. It has been demonstrated that *Nitrospirae* has a positive effect on promoting plant growth and enhancing the absorption of trace elements from soil to plants (Chen et al. 1981).

Li et al. (2014b) found that the fungal *Leptosphaerulina* sp., *Fusarium* sp. seemed to be the main cause for the yield decline and poor growth of monocultured peanut by root rot in the red soil. However, our previous experiment showed that peanut bacterial wilt caused by *R. solanacearum*, a devastating diseases for peanut production, had a ratio of peanut wilt was as higher as 58 % in L-CF treatments (Wang et al. 2011). According to the field pyrosequencing investigation the soil-borne disease *Ralstonia* sp. occurred in all treatments with its relative abundance under all treatments quickly increasing during the plant growth stage (Fig. 3). This increase may be due to no viable host being present for the pathogen at sowing time, with the increase in population associated with greater plant roots and exudates. The relative abundance of *Ralstonia* sp. in L-CF treatment was significantly higher than that in L-OM in August (Fig. 3). Many studies demonstrated that the organic fertilizer can be used for improving crop production, soil health, nutrient levels, organic matter, plant growth and suppression of disease caused by soil-borne plant pathogens (Chaney et al. 1980; Mays and Giordano 1989; Mikhail et al. 2005).

An abundance of beneficial soil organisms could suppress pathogens and diseases, improve nutrient availability, promote plant growth, and thus increased the crop yield (Ji et al. 2008; Ramesh et al. 2009; Liu et al. 2012; Yuan et al. 2014). *Streptomyces* sp. are especially prolific as plant growth promoters and fungal plant pathogen antagonists, improving crop yield by increasing photosynthesis, controlling soil diseases and accelerating decomposition of lignin material in the soil (Oskay

et al. 2005; Lwin and Ranamukhaarachchi 2006; Hill et al. 2011). *Streptomyces* sp. increased from 0.11, 0.12, and 0.23 % (April) to 0.45, 0.28, and 0.53 % (August) in S-CF, L-CF and L-OM treatments, respectively (Fig. 4). Additionally, the relative abundances of genera identified as *Bacillus* sp., *Arthrobacter* sp. and *Sphingomonas* sp., are now being used as worldwide bacterial antagonists against *R. solanacearum* (Li et al. 2005; Hu et al. 2007; Huang et al. 2011; Yuan et al. 2014). All of the three genera were increased in L-CF and L-OM treatments (Fig. 4). Interestingly, these genera were always higher in L-OM than in L-CF at the harvest time (Fig. 4). CCA further demonstrated that the beneficial microbial' abundance, such as *Arthrobacter*, *Bacillus* and *Sphingomonas* species, were positively correlated with the soil physicochemical properties, particularly pH and total and available P for the L-OM samples while the pathogenic bacterial, *R. solanacearum*, was negatively correlated with them. Similar results are also reported in the literature that the microbial maximal growth activity was observed in the long-term P fertilized soils (Zheng et al. 2009; Lin et al. 2012). We suspected that the organic fertilizers more effectively increased diversity (Table 2) and changed the community structure (Fig. 5) that may have increased competition between the bacterial community and the *R. solanacearum* pathogen than in the chemical fertilizer. Additionally, organic matter has many benefits for the soil food web as well as physical benefits to soil structure.

Conclusion

In summary, with the increasing years of continuous peanut monocropping, the peanut yield and height decreased. Soil pH and the contents of organic matter, N, P and K in the soils increased with the long-term fertilization compared with the 2-year of short-term chemical fertilization and the total contents of these nutrients were higher in the L-OM treatment than in the L-CF treatment. Pyrosequencing results clearly showed that bacterial community diversity under the long-term monocropping with chemical fertilizer was less than under organic fertilizer treatment during the sampling times. The bacterial pathogen *R. solanacearum* was found to cause peanut wilt disease at the late growing stage that lead to production decrease. The greater population of *R. solanacearum*, in the chemical fertilizer treatment resulted in more severe cropping obstacle, which could explain for yield declines over consecutive peanut monoculturing. The application of organic fertilizer was more beneficial for ameliorating the peanut continuous cropping obstacle than the chemical fertilizer. It may be due to the organic fertilizer has changed the microbial community to increase diversity and beneficial bacteria, which may have increased competition between the bacterial community and the *R. solanacearum* pathogen, as well as improving organic matter which has many benefits for the soil food web as well as physical benefits to soil structure.

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