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Bacterial community in peanut soils in various cropping systems

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ABSTRACT

We investigated (i). the composition of soil bacterial communities in continuous monoculture and rotation in 3-cropping systems (continuous maize monoculture for 3 years, continuous peanut monoculture for 3 years, maize - peanut rotation), (ii). if soil bacterial communities in the rhizosphere were affected by the addition of chemical soil amendments (mixture of biochar and silicon powder) and biological soil amendments (Kuai-rui-song) in continuous monoculture peanut. The Analysis of the V4 16S rRNA gene region on the Illumina MiSeq platform, identified the changes in bacterial diversity and community structure. Although the composition of predominant taxa was similar in all 5-treatments, but there were few differences in the least-abundant phyla such as Synergistetes, Tenericutes, GAL15 and WS2. There were four unique phyla [Synergistetes, GAL15, WS2 and Kazan-3B-28] in peanut fields in continuous monoculture. Four phyla [Tenericutes, WS2, OP3 and FCPU426] were significantly different ($p < 0.05$) in the peanut monoculture field and in the maize-peanut rotation field. Continuous monoculture with peanut had the highest bacterial community richness, as indicated by high Chao index and Ace index. In maize-peanut rotation, the rhizosphere bacteria belonged to 500 genera. Among the bacteria, *Bradyrhizobium*, *Rhodospirillum*, *Burkholderia*, *Candidatus Koribacter*, *Candidatus*, *Solibacter* and *Koribacteraceae* were more frequent. Although there was no improvement in bacterial diversity at the generic level, but the addition of soil amendments slightly altered the bacterial diversity than in peanut monoculture and the addition of biological soil amendment was slightly better than the chemical additive.

Keywords: Amendments, *Archis hypogea*, bacterial diversity, bacterial communities, continuous cropping, crop rotation, illumina sequencing, maize, monoculture, peanut, soil microbes, *Zea mays*.

INTRODUCTION

Due to the land scarcity in China, peanut (*Arachis hypogaea* L.) is increasingly grown as continuous monoculture crop. This has become a serious problem as it not only affects the crop development but also changes the composition of the soil microbial community (34), decline in yield and quality, increases the plant diseases and poor soil quality (17,25).

The soil microbial community is an integral component of soil quality, because of its crucial involvement in many processes (18). Many factors contribute to the problems in

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continuous cropping, but shif**ts** in soil microbial communities are the most important (28,53). The importance of soil microbial diversity for ecosystem functioning is known, but relatively little is known about how the monoculture affects the soil microbial community composition. Based on 18S rRNA gene clone libraries (10) and confirmed by pyrosequencing (29), it has been found that continuous monoculture of peanut affects the soil microbial communities. Bacterial communities over 4-peanut cropping cycles were assessed using the ribosomal intergenic spacer analysis (RISA) combined with 16S rRNA cloning and sequencing (48). These studies have shown that the complex interactions among the soil bacteria were important to crop health. However, most previous studies on the effects of continuous monoculture on the soil microbial communities were assessed by conventional isolation, culture and traditional molecular methods such as the Sanger sequencing-based analysis of 16S rRNA gene libraries or fingerprinting methods (32). These approaches have limitations of only analyzing relatively small number of clones and few different soil samples. The advent of high-throughput sequencing of 16S rRNA gene fragments has broadened the scope for systematic and comprehensive studies of soil microbial communities (1, 30, 31, 39).

In this study, we used the high-throughput DNA sequencing of the V4 16S rRNA gene region on the Illumina MiSeq platform to analyze differences in the bacterial community structure between 3-cropping systems. This research aimed to qualitatively and quantitatively measure the effects of different cropping system on the soil microbial community structure in rhizosphere and to know if continuous peanut monoculture alters the composition of soil bacterial communities than peanut-maize rotation.

MATERIALS AND METHODS

I. Sample collection

Continuous monoculture and rotation experiments with peanuts were done from 2011 to 2013 at the Peanut Scientific Research centre, Shenyang Agricultural University, Shenyang, China (123°33′ E, 40°48′ N, temperate humid and semi-humid monsoon climate, frost-free period : 148 to 180 d, annual rainfall : 547 mm and the mean annual temperature is 7.0-8.1°C). The field soil was Mottlic Hapli-Udic Argosols, according to the WRB-FAO classification (50), commonly called dry brown soil in China. The soil characteristics of this field before commencement of the tests were determined as per Bao (2). The soil organic matter (OM) was 13.1 g/kg, total nitrogen (TN) was 0.53 g/kg, total phosphorus (TP) was 0.67 g/kg, total potassium (TK) was 18.8 g/kg, available nitrogen (AN) was 56.2 mg/kg, available phosphorus (AP) was 12.5 mg/kg, available potassium (AK) was 89.6 mg/kg and the pH was 6.81.

The crop season was May to October, hence, one crop was grown per year, planting in May and harvesting in October. The experimental treatments were: (i). continuous maize monoculture for 3 years (MM), (ii). continuous peanut monoculture for 3 years (PM), (iii). maize - peanut rotation (MPR) for 3 years, (iv). continuous peanut monoculture for 3 years + chemical soil amendments (PMC), (v). continuous peanut monoculture for 3 years +

biological soil amendments (PMB). Each treatment was replicated thrice in Randomized block design. Each plot was 6×4.5 m. The dates of crop sowing and crop harvesting are shown in Table 1.

Table 1. Dates of sowing and harvest of 3-Years cropping Systems

S. No.	Cropping System	Crop Density (hole/ha)	Year I (2011)			Year II (2012)			Year III (2013)		
			Dates of								
			Crop	Sowing	Harvest	Crop	Sowing	Harvest	Crop	Sowing	Harvest
1	MM	67000	maize	May 4	Oct 3	maize	May 3	Oct 3	maize	May 5	Oct 5
2	PM	150000	peanut	May 4	Oct 3	peanut	May 3	Oct 3	peanut	May 5	Oct 5
3	MPR	67000/ 150000	peanut	May 4	Oct 3	maize	May 3	Oct 3	peanut	May 5	Oct 5
4	PMC	150000	peanut	May 4	Oct 3	peanut	May 3	Oct 3	peanut	May 5	Oct 5
5	PMB	150000	peanut	May 4	Oct 3	peanut	May 3	Oct 3	peanut	May 5	Oct 5

MM: continuous maize monoculture; MPR: rotation of maize and peanut; PM: continuous peanut monoculture; PMC: continuous peanut monoculture with chemical amendments; PMB: continuous peanut monoculture with biological amendments

The peanut cultivar used was 'HuaYu 33' and the Maize cultivar was 'ZhengDan 958'. Field management practices were the same for all experimental plots. Before peanut sowing, each plot was fertilized with 82.5 N kg/ha, 82.5 P_2O_5 kg/ha, and 97.5 k_2O kg/ha. All fertilizers were applied at sowing. The hole seeding/Dibbling method was used for sowing. The row spacing was 30 cm and the plant to plant distance was 15 cm. At maize sowing, 248 N kg/ha, 75 P_2O_5 kg/ha, and 65 k_2O kg/ha were applied as base fertilizer and 334 N kg/ha was top dressed during the V12 stage. The seeding method was hole seeding, the row spacing was 40 cm and plant to plant distance was 25 cm. Two amendments were added before sowing (i). Chemical soil amendment [mixture of biochar and silicon powder (1:1 Ratio)]. Biochar was produced by burning corn cobs at 450℃ and passing through 80-100 mesh sieve, (ii). Biological soil amendment, was biological improvement agent called 'Kuai-rui-song' from Wofengyuan Biotechnology Co., Ltd., China.

Soil samples were collected during the flowering of peanut on July 15, 2014. Each rhizosphere soil sample was collected by removing 5- plants at random from each plots and the roots were shaken gently to remove the loosely adhering rhizosphere soil. It was also carefully collected from fine roots by gently brushing the adhering soil with brush. Soil samples were homogenized, passed through < 2-mm sieve to remove plant materials, roots and stones, pooled, mixed and stored at 4°C for further analyses.

II. DNA extraction, PCR and high-throughput DNA sequencing

Total soil DNA was extracted from 15 soil sub-samples using the Mag-Bind® Soil DNA Kit (OMEGA Bio-Tek, Inc., Norcross, GA, USA) according to the manufacturer's instructions. Pyrobest DNA Polymerase (Takara, DR500A) was used to amplify the V4 hypervariable region of 16S rRNA gene from bacterial genome DNA using the degenerate primer (forward primers: 5' AYTGGGYDTAAAG NG 3', reverse primers: 5' TACNVGGGTATCTAATCC 3'). Polymerase chain reaction (PCR) system contained 5 × Q5 reaction buffer, 5 × Q5 GC high enhancer, 2.5 mM dNTPs, 10 μM of each primer, 5 U Q5 polymerase and 1 μL DNA template in final volume of 25 μL. The program was run at

98 °C for 30 sec to denature the DNA, with 25 cycles at 98 °C for 30 sec, annealing at 50 °C for 30 sec, extension at 72 °C for 30 sec, and then a final extension of 5 min at 72 °C. The PCR product was taken from 0.8 % agarose gel and purified using an Axyprep DNA Gel Extraction Kit (Axygen, AP-GX-500). V4 amplicons were sequenced using the pair-end method by Illumina MiSeq. The average length of sequence reads was 224 bp. Sequence reads were trimmed so that the average Phred quality score for each read was above Q20 and its length was > 150 bp. After trimming, these reads were assembled by Flash software (http://www.genomics.jhu.edu/software/FLASH/index.shtml) and the reads that could not be assembled were discarded. Only those reads with consecutive and identical base shorter than 5 bp, without ambiguous bases, were used for subsequent analysis. Chimera sequences were identified and removed using UCHIME (15). Sequences clustering was done by uclust (QIIME) with a similarity cutoff of 97%, and clustered into operational taxonomic units (OTUs). The longest sequence in each cluster was chosen as the representative sequences, which were blasted with the Green genes reference library (33).

III. Statistical analysis

The rarefaction analysis based on Mothur v.1.21.1 (42) was conducted to reveal the diversity indices, including the abundance-based coverage estimator (Ace), Chao l estimator (Chao l), Shannon diversity index (Shannon), Simpson diversity index (Simpson) and Good's library coverage. For all parameters, one-way analysis of variance (ANOVA) with Duncan and LSD multiple range tests and one-sample T-test were done for multiple comparisons using SPSS v20.0 (IBM SPSS Statistics for Windows, IBM Corp, Armonk, NY). Venn diagrams were constructed to visualize shared and unique OTUs between samples and performed in Vegan packages in R (38). For β-diversity, a heat map was created using the package pheatmap in R, based on the most abundant 50 OTUs through all samples.

RESULTS AND DISCUSSION

Sequence data and bacterial taxonomic richness

Total bacterial communities were characterized by high-throughput sequencing of rhizosphere soils in triplicate. Across all 15 sub-samples, a total of 1,021,059 high-quality sequences were obtained and grouped into 6144 OTUs at the 97% similarity cut-off level, after removal of the low quality reads. Among these high quality sequences, more than 99% ranged between 220 and 230 bp. An average of 101510, 70640, 76025, 51180 and 40997 bacterial sequences were obtained from the soil samples in MM, MPR, PM, PMC and PMB fields. The rarefaction curves of bacterial communities at 97% sequence similarity level in the 15 soils from fields are shown in Fig. 1. All amplified rarefaction curves increased rapidly from 0 to 10000 sequences and tended to plateau at 30000 sequence reads, which indicated that sequences derived diversity and richness in this study were sufficient to characterize the species in each samples.

The effective bacterial sequences in the 15 sub-samples were all assigned to corresponding taxonomies using BLAST combined with QIIME. From the phylum assignment results, it was found that few reads were classified as Archaea by

high-throughput sequencing, and 99.99% of the 16S rRNA sequence reads were identified as bacteria belonging to 40 phyla. The predominant 8 phyla were *Proteobacteria*, which averaged 25.3% relative abundance within all samples, followed by *Acidobacteria* 19.9%, *Actinobacteria* 14.5%, *Gemmatimonadetes* 10.2%, *Verrucomicrobia* 9.2%, *Chloroflexi* 5%, *Bacteroidetes* 5% and *Planctomycetes* with 5%. The remaining phyla were represented by 6% relative abundance of the total samples.

Alpha diversity was estimated by five indices: Chaol, ACE, Simpson, Shannon and Coverage (Table 2). It was found that the Chaol and ACE index values were different between MPR and the other treatments, which were the lowest in MPR. There were significant differences between PM and MPR by *t*-tests (Table 3). The values of these two indices significantly increased when peanut was monocultured (PM) and the values of Chaol and ACE in PM peaked at 4915.97 and 4957.32. The two indices of PMC and PMB dropped considerably in comparison to PM. The values of PMB declined significantly and arrived at 4380.31 and 4472.04. By *t*-tests, significant differences were noticed between PM and PMB. Conversely, the Shannon and Simpson diversity indices showed similar patterns to Chaol and ACE, but *t*-tests showed no significant differences between the treatments. Coverages were 97.11% and 98.73% for the sequence data sets from these five treatments, which suggested that the libraries represented the major bacterial phyla in the soils (Table 2).

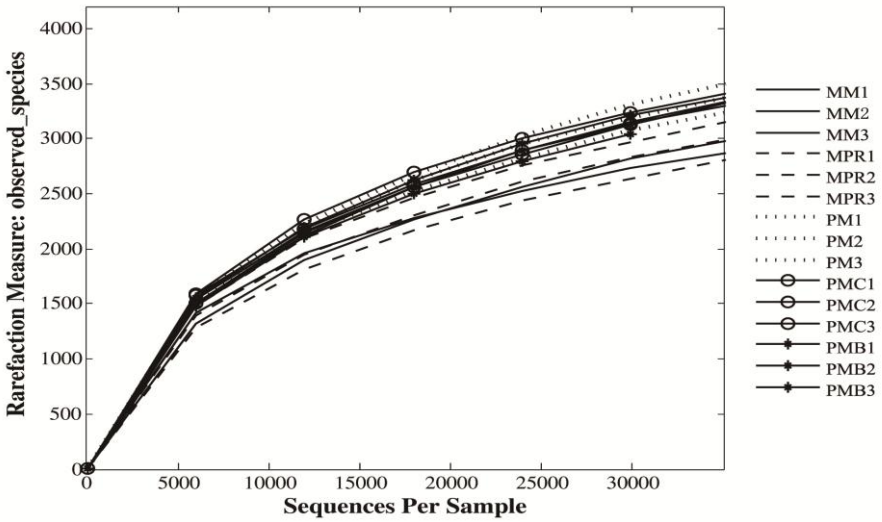

Figure 1. Rarefaction curves at 97% sequence similarity level of Rhizosphere soil samples various treatments

MM1, MM2, MM3: 3 repeats of consecutive maize monoculture; MPR1, MPR2, MPR3: 3 repeats of maize and peanut rotation; PM1, PM2, PM3: 3 repeats of consecutive peanut monoculture; PMC1, PMC2, PMC3: 3 repeats of consecutive peanut monoculture with chemical amendments; PMB1, PMB2, PMB3: 3 repeats of consecutive peanut monoculture with biological amendments

We have found that the Chaol and ACE indices were the highest in PM, but the Shannon and Simpson diversity indices showed no significant differences between the treatments. These results suggest that the community richness in continuous monoculture of peanut was higher than in other cropping systems. However, the community diversity had no significant differences in the five treatments because of lower community evenness in MPR, PMC and PMB. This is in accordance with the findings of Xiong *et al.* (51). Their results demonstrated that the long-term monoculture of vanilla significantly altered the soil fungal communities, whereas, the soil bacterial diversity was relatively stable.

Table 2. Bacterial α-diversity indices of rhizosphere soils from different treatments

Treatment	Chaol	ACE	Simpson	Shannon	Coverage (%)
MM	4755.22 ±298.17ab	4765.69 ±367.78ab	0.00625 ±0.0015a	6.44 ±0.098a	98.73 ±0.91a
MPR	4407.90 ±95.11b	4408.55 ±83.88b	0.00589 ±0.0020a	6.42 ±0.205a	98.53 ±0.36a
PM	4915.97 ±244.55a	4957.32 ±278.18a	0.00548 ±0.0018a	6.59 ±0.088a	98.56 ±0.33a
PMC	4579.01 ±304.12ab	4611.97 ±273.05ab	0.00569 ±0.0016a	6.57 ±0.110a	97.71 ±0.46a
PMB	4380.31 ±251.68b	4472.04 ±240.17ab	0.00506 ±0.0006a	6.57 ±0.015a	97.11 ±0.48a

Different letters indicate significant differences among treatments at the 5% level

Chaol, ACE: Community richness indexes; Simpson, Shannon: Community diversity indexes; Coverage: Sequencing depth index; MM: Continuous maize monoculture; MPR: Maize peanut rotation; PM: Continuous peanut monoculture; PMC: Continuous peanut monoculture with chemical amendments; PMB: Continuous peanut monoculture with biological amendments.

Table 3. ANOVA for Bacterial α-diversity indices in rhizosphere soils from different treatments.

Dependent variable	(I)num	(J)num	Standard error	Sig.	95% confidence interval	
					upper	lower
Chaol	MPR	PM	204.481	0.032*	-963.679	-52.453
	PM	PMB	204.481	0.026*	80.039	991.264
ACE	MPR	PM	216.617	0.030*	-1031.419	-66.115
	PM	PMB	216.617	0.049*	2.627	967.931

*$p < 0.05$; **$p < 0.01$, Chaol, ACE: community richness indexes

MM: Continuous maize monoculture; MPR: Maize peanut rotation; PM: Continuous peanut monoculture; PMC: Continuous peanut monoculture with chemical amendments; PMB: Continuous peanut monoculture with biological amendments. I, J:Two dependent samples.

Bacterial community composition

The structures of bacterial communities in all subsamples from the five treatments were compared at the level of bacterial phyla level (Fig. 2). The overall bacterial composition of different treatments was similar, while the distribution of each phylum or group slightly varied. *Proteobacteria* was the dominant phylum across all samples accounting for 23.2-27.8% of total 16SrRNA gene reads sequences. The relative abundance of *Acidobacteria* was second, with an average abundance of 23.8%, 20.8%, 21.2%, 16.4% and 17.3% in the MM, MPR, PM, PMC and PMB treatments. Minor numbers of diverse

phyla were observed in all samples, including *Cyanobacteria* (0.6%), *Firmicutes* (0.6%), *Armatimonadetes* (0.4%), *Nitrospirae* (0.4%), *Tenericutes* (0.2%), *Euryarchaeota* (0.1%) and *Chlorobi* (0.1%). In addition, many sequences were assigned as candidate phyla to TM7 (1.1%), OD1 (0.9%), WS3 (0.3%), AD3 (0.2%) and FBP (0.1%). The described distribution of bacterial phyla in the soil supports the results from other soils (22), where the majority of 16S rRNA gene soil clone libraries belonged to 9 major bacterial phyla. At both times of soil sampling *Proteobacteria* was dominant, followed by *Acidobacteria*. This result is in agreement with previous studies of soil agro-ecosystems (14,16,46,48).

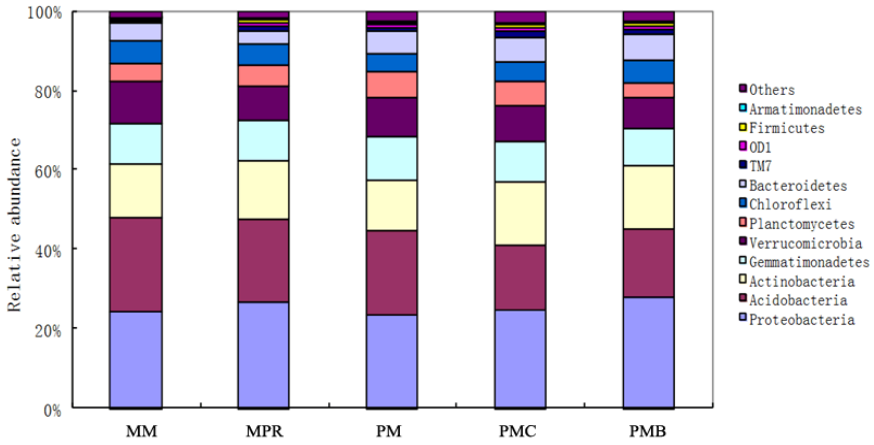

Figure 2. Relative abundances of main bacterial phyla in Rhizosphere soils under different treatments

MM: Consecutive maize monoculture; MPR: Rotation of maize and peanut; PM: Consecutive peanut monoculture; PMC: Consecutive peanut monoculture with chemical amendments; PMB: Consecutive peanut monoculture with biological amendments

Although the composition of predominant taxa was similar in the five treatments, there were some differences in the least-abundant phyla. The phylum assignment results showed that the rhizosphere bacteria belonged to 36 phyla in the MPR treatment. *Acidobacteria*, *Actinobacteria*, *Gemmatimonadetes*, *Proteobacteria* and *Verrucomicrobia* were represented by 81% of the total sample relative abundance, and the remaining 31 phyla were represented by 19%. *Proteobacteria* accounted for the largest proportion with 20.8% among all phyla. Compared with MPR, the rhizosphere bacteria in MM and PM belonged to 40 phyla. There were 36 of the same phyla as in MPR and four additional unique phyla (*Synergistetes*, GAL15, WS2 and Kazan-3B-28). Similar to the above treatments, the rhizosphere bacteria belonged to 38 phyla, and there were 36 of the same phyla as in MPR and two additional unique phyla (Kazan-3B-28 and FCPU426) in PMC. Kazan-3B-28 and GAL15 were unique in PMB. A detailed comparison of these phyla is shown in Table 4. In addition, the ANOVA results show that among the phyla, only four were significantly different ($p < 0.05$) between PM and MPR (Table 5). Of these four, *Tenericutes*, WS2 and

Table 4. Comparison of number of sequences of all phyla from Rhizosphere soil samples in various treatments**.**

Taxon	MM	MPR	PM	PMC	PMB
No blast	114	455	39	49	30
Crenarchaeota	24	28	64	64	35
Euryarchaeota	240	151	252	171	117
Parvarchaeota	1	4	15	2	2
Bacteria ;p_____	59	69	37	19	12
AD3	176	715	440	162	125
Acidobacteria	66836	41954	45663	23585	20011
Actinobacteria	38024	29540	27977	22750	18128
Armatimonadetes	855	890	862	649	601
BHI80-139	16	4	11	14	10
BRC1	103	34	73	65	60
Bacteroidetes	12611	6553	12604	9044	7463
Chlamydiae	46	97	74	40	27
Chlorobi	258	146	182	161	141
Chloroflexi	16279	10137	9781	6687	6399
Cyanobacteria	365	592	909	1674	1114
Elusimicrobia	6	31	29	26	9
FBP	198	167	179	179	112
FCPU426	2	12	3	0	1
Fibrobacteres	108	85	64	52	15
Firmicutes	949	1512	1032	1070	868
GAL15	13	0	2	2	0
GN02	4	4	14	16	11
Gemmatimonadetes	28665	20232	23676	15070	11002
Kazan-3B-28	4	0	3	0	0
Nitrospirae	1954	267	1412	462	428
OD1	1189	2132	2127	1517	914
OP11	6	11	23	9	6
OP3	72	38	120	111	66
Planctomycetes	11751	10966	14086	8635	4473
Proteobacteria	67656	53943	50140	35211	32064
Synergistetes	2	0	12	1	1
TM6	34	24	31	16	18
TM7	1043	2533	1506	2351	1651
Tenericutes	51	202	411	356	244
Thermotogae	44	1	7	37	15
Verrucomicrobia	30159	17741	20602	13013	8874
WPS-2	90	251	145	72	32
WS2	4	0	8	1	4
WS3	731	222	954	386	265
Thermi	32	10	33	37	14

MM: Consecutive maize monoculture; MPR: Rotation of maize and peanut; PM: Consecutive peanut monoculture; PMC: Consecutive peanut monoculture with chemical amendments; PMB: Consecutive peanut monoculture with biological amendments

OP3 were significantly higher in PM. Conversely, FCPU426 was significantly lower in PM. Research shows that Tenericutes was the least-abundant phylum in the rhizosphere of Lolium (24). Little is known about bacterial groups with low abundance and activity in soils

because most of these phyla have not yet been studied and their role in soils and rhizosphere remain unknown. Sengupta and Dick (43) reported that Tenericutes only dwelt in plow-till plots and were not present in no-till plots. They suggested that plowing and secondary tillage tended to homogenize the soil and reduce the diverse and unique micro environments in which microbial populations reside. Generally it is believed that continuous monoculture tends to homogenize the soil and hence a similar pattern is evident on plowing and secondary tillage, and the abundance of Tenericutes increases. We feel that the Tenericutes may be considered as indicator bacteria of homogeneous soils, which may be possible factor in causing the continuous cropping obstacle after consecutive monoculture of peanut.

Table 5. Analysis of variance for phylum level in rhizosphere soils from various treatments

Dependent variable	(I)num	(J)num	Standard error	Sig.	95% confidence interval	
					upper	lower
Tenericutes	MPR	PM	30.472	0.045*	−137.56	−1.77
WS2	MPR	PM	1.174	0.046*	−5.28	−0.05
OP3	MPR	PM	7.223	0.004**	−43.43	−11.24
	MPR	PMC	7.223	0.007**	−40.43	−8.24
FCPU426	MPR	PM	0.789	0.003**	1.24	4.76
	MPR	PMC	0.789	0.000**	2.24	5.76
	MPR	PMB	0.789	0.001**	1.91	5.42

*$p < 0.05$; **$p < 0.01$

MM: Consecutive maize monoculture; MPR: Rotation of maize and peanut; PM: Consecutive peanut monoculture; PMC: Consecutive peanut monoculture with chemical amendments; PMB: Consecutive peanut monoculture with biological amendments. I, J:Two dependent samples.

The results showed that the rhizosphere bacteria belonged to 500 genera in MPR. Further, there were 538, 537, 534 and 517 genera in MM, PM, PMC and PMB. In addition, at the generic level, comparison of the relative abundances revealed slight differences in the soil bacterial communities of the five fields (Table 6). Among the genera, *Bradyrhizobium*, *Rhodospirillum* and *Burkholderia* belonged to *Proteobacteria* and were more frequent in MPR than in other continuous monoculture field samples. In addition, there was a similar pattern in the genera *Candidatus*, *Koribacter*, *Candidatus*, *Solibacter* and *Koribacteraceae*, which belonged to *Acidobacteria*. These results indicate a higher sensitivity to rotation of the following six genera or families: *Bradyrhizobium*, *Rhodospirillum*, *Burkholderia*, *Candidatus*, *Koribacter*, *Candidatus*, *Solibacter* and *Koribacteraceae*. Rhizobia nodulating peanut are slow-growing Bradyrhizobia and described as *Bradyrhizobium* spp.. Strains of Bradyrhizobia have miscellaneous host specificity and outstanding ecological adaptability because they not only inhabit the soil and rhizosphere, but also aquatic ecosystems and nodulate *Aeschynomene* species (45,49). These bacteria can nodulate legumes, non-legume *Parasponia andersonii* as the nitrogen fixing endosymbionts (5,21,37), or even in rice as the endophytic bacteria (9). Bradyrhizobia and rhizobia are symbiotic bacterial partners in nitrogen fixing nodules on legumes.

Until recently, it had been generally accepted that legumes and the non-legume of the genus *Parasponia* were nodulated exclusively by members of the family *Rhizobiaceae* in

the α-proteobacteria, which included the genera *Bradyrhizobium*, *Azorhizobium*, *Rhizobium* and others (47). In the past decade, however, several other species of α-proteobacteria have been shown to nodulate legumes (35). A few members of the β-proteobacteria, such as *Burkholderia* spp. have also been reported in nodules of tropical legumes (36). *Burkholderia* species are effective symbionts of several plants species (6,7,11,12). Although some are important pathogens of humans and animals (e.g., *Burkholderia cepacia*, *B. pseudomallei* and *B. mallei*), studies have shown that the symbiotic species are phylogenetically distant from these pathogenic species (8). Burkholderia species have also been reported to exhibit direct or indirect mechanisms as plant growth promoters and biological control agents (13,19,44).

Table 6. Comparison of OTU numbers at the genus level in rhizosphere soils from different treatments

Phylum	Genus	OTUs				
		MM	MPR	PM	PMC	PMB
Proteobacteria	*Bradyrhizobium*	1894	2438	2297	1523	1114
	Rhodospirillum	1278	1548	1394	785	814
	Burkholderia	749	1095	789	612	526
	Total	**3921**	**5081**	**4480**	**2920**	**2454**
Acidobacteria	*Candidatus Koribacter*	1102	2183	1856	692	569
	Candidatus Solibacter	742	1623	1182	848	590
	Koribacteraceae	2954	4953	3729	1484	1299
	Total	**4798**	**8759**	**6767**	**3024**	**2458**

OTUs: operational taxonomic units

MM: Consecutive maize monoculture; MPR: Rotation of maize and peanut; PM: Consecutive peanut monoculture; PMC: Consecutive peanut monoculture with chemical amendments; PMB: Consecutive peanut monoculture with biological amendments.

Acidobacteria is the second most abundant phylum identified in this study, and it has also been found in diverse soil types from North to South America (23,26), active in cultivated wheat associated with later stages of the 'take-all decline' disease (41), in the rhizosphere of chestnut trees and peanuts (20,27). Available data suggest that members of the phylum Acidobacteria are genetically and metabolically diverse, environmentally widespread and perhaps as ecologically important as the well-known Proteobacteria and gram-positive bacterial phyla (3,4,22). Acidobacteria groups sensitive to some cultural practices may be considered as important indicators of soil quality (40). Acidobacteria group 4 has been shown to be enhanced in wheat-soybean rotation (52). This view is consistent with the results of the present study that *Candidatus*, *Koribacter*, *Candidatus*, *Solibacter* and *Koribacteraceae* populations increased in the maize-peanut rotation system. From the above analysis, we deduce that such increases in bacteria in the maize-peanut rotation system are important for the promotion of plant growth and maintenance of soil quality.

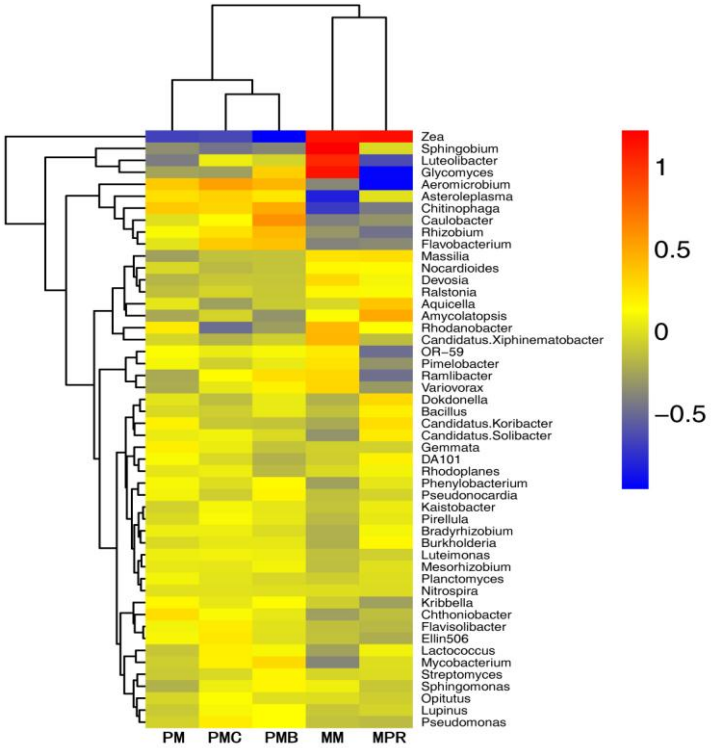

Figure 3. Heat map representing the most abundant 50 OTUs through all samples.
The heat map plot depicts the relative abundance of each bacterial genera with each sample. The color code grading from red to blue in map, indicates the relative abundances from the highest to the lowest.

To better visualize the patterns of occurrence and abundance of the predominant bacterial genera of this soil as affected by five cropping systems, the most abundant 50 OTUs which were annotated to genus were compared and clustered using the heatmap (Fig. 3). This heatmap showed that five rhizosphere soils were divided into two groups, with MPR and MM clustered together, but separate from PM, PMC and PMB. These cluster results suggest that bacterial diversity is to some extent, associated with crop species. The MM and MPR treatments with maize, had high percentages of *Zea* and low percentages of *Aeromicrobium*, *Asteroleplasma*, *Chitinophaga*, *Caulobacter*, *Rhizobium* and *Flavobacterium* compared with the PM, PMC and PMB treatments. Additionally, this heat map also shows that the Rhizosphere soil of PM had the largest difference from MPR. Although the bacterial diversity of PMB and PMC was similar to PM, there were some slight differences between them. The Venn diagram (Fig. 3) also shows that the number of unique OTUs in MPR was lower than in the other treatments. However, the pattern in the number of bacterial unique OTUs were in accordance to the results of Xiong *et al*. (51), who showed that unique OTUs declined over successive years of vanilla monoculture. In

addition, more bacterial unique OTUs were found in healthy soil than in diseased potato soils in Michigan, USA (40). Venn diagrams were generated using R based on the share OTU tables from the five field samples (Fig. 4). For bacteria, 1989, 1038, 1967, 1733 and 1510, their unique OTUs were present in the MM, MPR, PM, PMC and PMB fields respectively, and 3663 shared OTUs were observed across all five treatments.

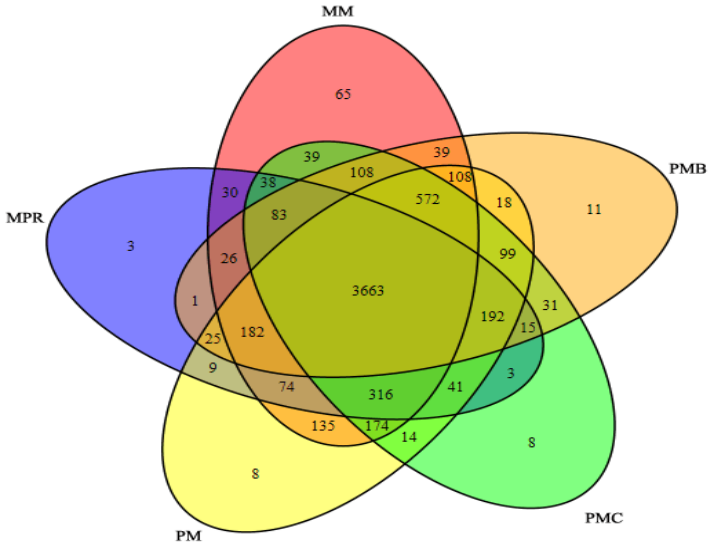

Figure 4. Venn diagram indicating the unique and shared observed operational taxonomic units of rhizosphere soil samples under different treatments.
MM: Consecutive maize monoculture; MPR: Rotation of maize and peanut; PM: Consecutive peanut monoculture; PMC: Consecutive peanut monoculture with chemical amendment; PMB: Consecutive peanut monoculture with biological amendment

CONCLUSIONS

Although the composition of predominant taxa was similar in all 5-treatments, there were few differences in the least-abundant phyla such as Synergistetes, Tenericutes, GAL15, WS2 etc. There were four unique phyla [Synergistetes, GAL15, WS2 and Kazan-3B-28] in the soils of continuous peanut monoculture. Four phyla [Tenericutes, WS2, OP3 and FCPU426] were significantly different in the peanut monoculture soil and in the maize-peanut rotation field soil. Continuous monoculture with peanut had the highest bacterial community richness, as indicated by high Chao index and Ace index but the community diversity had no significant differences in the five treatments because of lower community evenness. At the generic level, our results suggest that the bacterial diversity was strongly associated with the crop species to some extent and also affected by the cropping system. Rotation of maize and peanut significantly increased the abundance of several genera. The Genera, such as *Bradyrhizobium*, *Rhodospirillum*, *Burkholderia*,

Candidatus, *Koribacter*, *Candidatus*, *Solibacter* and *Koribacteraceae* were stimulated. Although the improvement effect was not found, bacterial diversity was slightly altered by addition of soil amendments compared with PM. The effect of chemical soil amendment was slightly better than the biological additive. However, the primary purpose of our study was to explore if maize-peanut rotation would lead to some changes at generic level. This study is an initial step towards developing a better understanding of the effects and interactions of monoculturing peanut on soil microbial communities, and the distribution and activity of these changing bacterial groups in rhizosphere microsites. Their roles in soil nutrient cycling and plant growth remain for future investigation.

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