Long-term continuously monocropped peanut significantly changed the abundance and composition of soil bacterial communities

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Soil sickness is the progressive loss of soil quality due to continuous monocropping. The bacterial populations are critical to sustaining agroecosystems, but their responses to long-term peanut monocropping have not been determined. In this study, based on a previously constructed gradient of continuous monocropped plots, we tracked the detailed feedback responses of soil bacteria to short- and long-term continuous monocropping of four different peanut varieties using high-throughput sequencing techniques. The analyses showed that soil samples from 1- and 2-year monocropped plots were grouped into one class, and samples from the 11- and 12-year plots were grouped into another. Long-term consecutive monocropping could lead to a general loss in bacterial diversity and remarkable changes in bacterial abundance and composition. At the genera level, the dominant genus *Bacillus* changed in average abundance from 1.49% in short-term monocropping libraries to 2.96% in the long-term libraries. The dominant species *Bacillus aryabhattai* and *Bacillus funiculus* and the relatively abundant species *Bacillus luciferensis* and *Bacillus decolorationis* all showed increased abundance with long-term monocropping. Additionally, several other taxa at the genus and species level also presented increased abundance with long-term peanut monocropping; however, several taxa showed decreased abundance. Comparing analyses of predicted bacterial community functions showed significant changes at different KEGG pathway levels with long-term peanut monocropping. Combined with our previous study, this study indicated that bacterial communities were obviously influenced by the monocropping period, but less influenced by peanut variety and growth stage. Additionally, soil pH, available P, K could be closely related with soil bacterial communities under long-term monocropping of peanut. Some

bacterial taxa with increased abundance have functions of promoting plant growth or degrading potential soil allelochemicals, and should be closely related with soil remediation and may have potential application to relieve peanut soil sickness. Simplification of bacterial communities, especially beneficial communities, and bacterial community function simplification with long-term peanut monocropping could be the main cause of peanut soil sickness.

Long-Term Continuously Monocropped Peanut Significantly Changed the Abundance and Composition of Soil Bacterial Communities

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ABSTRACT

Soil sickness is the progressive loss of soil quality due to continuous monocropping. The bacterial populations are critical to sustaining agroecosystems, but their responses to long-term peanut monocropping have not been determined. In this study, based on a previously constructed gradient of continuous monocropped plots, we tracked the detailed feedback responses of soil bacteria to short- and long-term continuous monocropping of four different peanut varieties using high-throughput sequencing techniques. The analyses showed that soil samples from 1- and 2-year monocropped plots were grouped into one class, and samples from the 11- and 12-year plots were grouped into another. Long-term consecutive monocropping could lead to a general loss in bacterial diversity and remarkable changes in bacterial abundance and composition. At the genera level, the dominant genus *Bacillus* changed in average abundance from 1.49% in short-term monocropping libraries to 2.96% in the long-term libraries. The dominant species *Bacillus aryabhattai* and *Bacillus funiculus* and the relatively abundant species *Bacillus luciferensis* and *Bacillus decolorationis* all showed increased abundance with long-term monocropping. Additionally, several other taxa at the genus and species level also presented increased abundance with long-term peanut monocropping; however, several taxa showed decreased abundance. Comparing analyses of predicted bacterial community functions showed significant changes at different KEGG pathway levels with long-term peanut monocropping. Combined with our previous study, this study indicated that bacterial communities were obviously influenced by the monocropping period, but less influenced by peanut variety and growth stage. Additionally, soil pH, available P, K could be closely related with soil bacterial

communities under long-term monocropping of peanut. Some bacterial taxa with increased abundance have functions of promoting plant growth or degrading potential soil allelochemicals, and should be closely related with soil remediation and may have potential application to relieve peanut soil sickness. Simplification of bacterial communities, especially beneficial communities, and bacterial community function simplification with long-term peanut monocropping could be the main cause of peanut soil sickness.

INTRODUCTION

Soil sickness is the progressive loss of soil quality due to continuous monocropping and results in the reduction of crop yield and quality, as well as a prevalence of soil-borne diseases (*Huang et al., 2013; Van der Putten et al., 2013*). It is a major problem in agriculture ecosystems all over the world, and has been reported for many types of crops, including food (e.g., rice, wheat, corn, soybean and peanut), economic (e.g., sugarcane and tobacco), vegetable (e.g., cucumber and eggplant) and medicinal crops (e.g., *Rehmannia*, ginseng and *Angelica*) (*Liu et al., 2012; Gentry et al., 2013; Huang et al., 2013; Wu et al., 2015*). Monocropping is considered unsustainable in agricultural systems; however, modern agricultural practices are often characterized by monocropping (*Cook, 2006*).

Based on previous reports, there are four main factors contributing to soil sickness: disorder in physicochemical soil properties, production and accumulation of autotoxins, imbalance of soil microbial communities and change in soil enzyme activity (*Huang et al., 2013; Zhou et al.,2018*). Soil microorganisms that are critical to many soil biological, chemical and physical processes

such as soil structure formation, mineral nutrition cycling, organic matter turnover and toxin accumulation or removal, are considered to be key drivers of terrestrial ecosystems (*Bever et al., 2012; Blagodatskaya and Kuzyakov, 2013*). Alteration of soil microbial communities can change the function performed by the communities and then feedback on plant growth and health (*Bever et al., 2012; Zhou et al., 2017*). In addition, the soil microbial community can serve as a sensitive bioindicator of soil health due to its quick response to environmental changes and close relationship with soil conditions and land management (*Sharma et al., 2010*). Consequently, understanding how soil microbial communities are affected by continuous monocropping is necessary to provide insights into soil sickness.

The bacterial populations are most abundant and diverse in soil, and the interactions between soil, bacteria and plant in roots related environments play key roles in soil fertility, sustainability and plant quality (*Chaparro et al., 2012*). Many bacterial taxa have been identified as biocontrol agents against soil-borne pathogens and play key roles in promoting plant growth, and some soil bacteria also have been reported as plant pathogens (*Compant et al., 2010; Santoyo et al., 2012; Buttimer et al., 2017*). Increasing evidence indicates that the soil bacterial communities can be shaped by plants through secretion of root exudates (*Doornbos et al., 2012*). Modifications in soil microbe populations induced by peanut (*Arachis hypogaea* L.) root exudates, rather than direct allelopathy, could contribute to peanut soil sickness (*Li et al., 2014a*). Additionally, recent studies suggested that accumulations of microbial pathogens at the expense of plant-beneficial microorganisms in the soil are likely explanations for yield declines as a consequence of consecutive monocropping (*Chen et al., 2012; Li et al., 2014b; Xiong et al.,*

85 *2015*). Therefore, clarifying changes in soil bacterial community properties in continuous
86 monocropping systems should be helpful for developing practices to relieve soil sickness in
87 agricultural production.

88 Peanut, an important oil and economic crop worldwide, is very adaptable to climatic
89 conditions and grows in tropical, subtropical and warm temperate climate regions across the
90 world. Due to limitations of arable land and requirements for developing regional agro-
91 industrialization, large-scale monocropping of peanut is common in China (*Chen et al., 2016*).
92 Research indicates that consecutive peanut monocropping has caused a decline in yield and
93 quality and increases in disease pressures (*Wang et al., 2005*). Early studies showed that soil
94 diversity and abundance of bacterial communities changed with continuous peanut
95 monocropping according to phospholipid fatty acid (PLFA), denaturing gradient gel
96 electrophoresis and library analyses (*Li et al., 2012; Chen et al., 2014; Liu et al., 2015*). Our
97 earlier study also indicated that the balance of soil bacterial communities was disturbed during
98 three years of continuous monocropping (*Chen et al., 2014*). However, the specific
99 characteristics of the soil bacterial community and the changes of soil bacterial structure and
100 composition in response to long-term peanut monocropping are unclear.

101 In this study, based on a gradient that we previously constructed of continuously monocropping
102 in a peanut field, we analyzed and compared responses of root soil bacterial communities of four
103 peanut varieties to monocropping for 1, 2, 11 and 12 years, using high-throughput sequencing
104 techniques. This study is to investigate bacterial community dynamics succession under long-
105 term peanut monocropping based on monocropping gradient experiment plots with a consistent

106 background. The aims of this study were to (i) determine the change characteristics of the soil
107 bacterial community and the influences of peanut varieties on the dynamics of the bacterial
108 community, under long-term continuous monocropping of peanut and (ii) identify the key
109 bacteria taxa related to peanut soil sickness.

110 **MATERIALS AND METHODS**

111 **Field experiment and soil sampling**

112 The field experimental site was set up in Laixi experimental farm at the Shandong Peanut
113 Research Institute, Qingdao, China (36° 50′ N, 120° 31′ E). A gradient of consecutive
114 monocropped peanut experiment plots that we previously developed was used. Independent
115 pools were applied, with the size of each pool being 4 m long, 1.5 m wide and 1 m high. We
116 collected soil from the plow layer of the cultivated land that had previously been planted with a
117 wheat-corn rotation, thoroughly mixed and then added to the pools. In order to construct the
118 gradient continuous monocropping plots, sweet potatoes were rotated with peanut in some pools
119 during the experiment. Peanuts were planted in May each year and harvested in October, all field
120 management, including the planting pattern and the use of water and fertilizer were consistent
121 among different pools. After harvest, the plots lay fallow until the next planting season. The
122 distance between rows was 16.5 cm and the ridge width was 70 cm, with two peanut seeds per
123 hole. Before planting, about 300 kg ha^{-1} of urea, 750 kg ha^{-1} of calcium magnesium phosphate,
124 and 225 kg ha^{-1} of potassium chlorate were used. The weeds were controlled using glyphosate
125 (41% active ingredient in 3 L ha–1) before planting. Plastic film mulching was applied during

126 cultivation. In addition, to minimize the influence of other factors including variety on the
127 measured indexes, the planting position of the same peanut variety was fixed during the
128 continuous cropping period. The longest-running pools have now been continuously
129 monocropped with peanuts for 16 years.

130 Combined with our previous study (*Jiao et al., 2015*), we selected four peanut varieties with
131 distinct responses to monocropping to analyze their soil bacterial community structure in plots
132 monocropped for 1, 2, 11 and 12 years. The four varieties included two large fruit varieties,
133 Huayu 917 and Huayu 50, and two small fruit varieties, Huayu 26 and Huayu 20. Based on
134 previous testing of their yield-related indexes, Huayu 20 was the most sensitive to long-term
135 monocropping, Huayu 917 was intermediate and Huayu 26 and Huayu 50 were tolerant. There
136 were three replicates for each treatment. The soils were collected at the full-bloom stage and five
137 randomly selected replicate test plants were used for each sample. The soils around individual
138 plants were sampled using a soil probe (1.5 cm diameter) at 5–10 cm soil depth, and at distances
139 of 3–5 cm, 8–10 cm, and 13–15 cm from the main root. The root zone soils were then mixed
140 together. These distances were chosen because the roots at this depth and these distances were
141 relatively abundant and the soil microbial community around the peanut root system could be
142 well characterized (Chen *et al*., 2012). Physichemical properties of pH, organic matter, avilable
143 N, P, K in the soil samples were determined using routine methods (Lu 1999).

144 **DNA extraction, pcr and high-throughput sequencing**

145 For each soil sample, total DNA was extracted with the Power Soil DNA Isolation Kit
146 (MoBio Laboratories Inc., Carlsbad, CA, USA) following the manufacturer's instructions. The
147 concentration and purity of the DNA was checked by electrophoresis on 1.0 % (w/v) agarose
148 gels. Soil bacterial communities were analyzed with amplicon sequencing on an Illumina HiSeq

platform. Two primers, 515F (5′- GTGCCAGCMGCCGCGGTAA-3′) and 907R (5′-CCGTCAATTCCTTTGAGTTT -3′), for the V4–V5 region of the 16S rRNA gene were applied. Both the forward and reverse primers had a 6-bp barcode unique to each sample. Each soil sample was independently amplified in triplicate, and the triplicate PCR reaction products for each sample were pooled and then all products were purified using a GeneJET^{TM} gel extraction kit (Thermo Scientific, Waltham, USA). The purified amplicons from each sample were pooled in equimolar concentrations and the mixture was then sequenced on an Illumina HiSeq platform at Novogene Co. Ltd, Beijing, China.

Sequencing data processing and statistical analysis

The paired-end reads from the original DNA fragments were merged with FLASH software (*Edgar, 2013*). The QIIME 1.7.0 software package was used to quality-filter and process the raw sequencing reads (*Caporaso et al., 2010*). The UCLUST method was used to delineate the operational taxonomic units (OTUs) at a threshold of 97% identity (*Caporaso et al., 2010*). A representative sequence for each OTU was taxonomically classified using the Silva Database (https://www.arb-silva.de/) based on Mothur algorithm (*Quast et al., 2013*). The OTU abundance data were normalized using a standard sequence number corresponding to the sample with the least sequences. The output normalized data were used for the subsequent analyses.

To assess the bacterial alpha-diversity of each sample, analyses including rarefaction curves, Shannon index and Chao1 index were performed using QIIME (Version 1.7.0) and displayed with R software (Version 2.15.3). Beta diversity analyses were used to estimate sample

differences in bacterial community compositions. In order to analyze the influence of peanut monocropping on soil bacterial community composition, heatmap analysis was performed with R software. Cluster analysis was preceded by principal component analysis (PCA), which was applied to reduce the dimensions of the original variables using the FactoMineR package and ggplot2 package in R software (Version 2.15.3). Principal coordinate analysis (PCoA) was performed to obtain principal coordinates and visualize the complex multidimensional data. The PCoA analysis was performed using the WGCNA, stat and ggplot2 packages in R software (Version 2.15.3). Unweighted pair-group method with arithmetic means (UPGMA) clustering was performed as a type of hierarchical clustering method to interpret the distance matrix using average linkage and was conducted using QIIME software (Version 1.7.0). Taxa with significantly diverse abundance with long-term monocropping at different levels were further investigated using the Metastats method and t-test using R software (Version 2.15.3). Tax4Fun functional prediction was achieved by the nearest neighbor method based on the minimum 16S rRNA sequence similarity. The data set was deposited in the NCBI-Sequence Read Archive with the submission Accession Number PRJNA559575.

RESULTS

Physichemical properties of soil

Physichemical properties of pH, organic matter, avilable N, P, K in the peanut monocropping soil were tested and analyzed (Table S1). The soil pH in samples from 2-year monocropped plots (7.20 - 8.05) were slightly higher than that in 1-year monocropped plots

189 samples (8.30 - 8.59), but obviously decreased in the samples from 11- and 12-year
190 monocropped plots (6.46 – 6.92). The contents of available P (15.12 – 21.12 mg kg^{-1}) in long-
191 term peanut monocropped plots soil were lower than that (39.37 - 48.58 mg kg^{-1}) in short-term
192 monocropping samples. The contents of available K (59.98 – 86.13 mg kg^{-1}) in long-term
193 monocropping samples were also lower than that (99.42 -120.67 mg kg^{-1}) in short-term
194 monocropping samples. But the contents of organic matter and available N didn't show obvious
195 changes with long-term monocropping of peanut.

196 **Characteristics of sequencing data**

197 In order to compare the soil bacterial community structure and composition of the four
198 peanut varieties to short- and long-term monocropping periods, a total of 48 16S rRNA gene
199 libraries were analyzed using high-throughput sequencing technique. The sampling variables,
200 including four peanut varieties and four monocropping periods, are shown in Table 1. A total of
201 2,541,421 quality-filtered sequences obtained from the 48 samples, ranging within 37,605–
202 68,117 with an average length of 373 bp, resulted in identification of a total of 9028 OTUs
203 applying a 3% sequence dissimilarity cutoff. After data normalization, 1,805,040 quality-filtered
204 sequences affiliated with 8856 OTUs were obtained. The bacterial complexity of the 48 samples
205 was estimated on the basis of alpha-diversity (OTU number, Chao1 index and Shannon index)
206 and showed relatively higher bacterial diversity in samples from the 1- and 2-year compared to
207 the 11- and 12-year monocropped plots (Table 1). Rarefaction curves (Figure S1) and indices of
208 richness and diversity including all samples' Chao1 and Shannon indexes (Table 1) tended to

209 approach a saturation plateau, indicating that the majority of bacterial diversity was recovered by
210 the surveying effort.

211 **Bacterial community structure and composition**

212 In total, 99.73% of the identified 8856 OTUs were related to bacteria, and the other 24
213 OTUs accounting for 0.15% of the total quality-filtered sequences were affiliated with Archaea.
214 There were 41 bacteria phyla detected across all samples and 0.73% of bacterial sequences were
215 unclassified at phylum level. The top 10 phyla accounted for 95.49% of the total sequences and
216 79.67% of the total OTUs. The dominant phyla across all samples were Proteobacteria,
217 Actinobacteria, Acidobacteria and Chloroflexi, accounting for 30.71, 21.59, 18.04 and 7.69% of
218 the total sequences, respectively, and 26.61, 7.43, 6.49 and 10.77% of the total 8856 OTUs,
219 respectively. The phyla Gemmatimonadetes, Firmicutes, Planctomycetes, Nitrospirae,
220 Bacteroidetes and Thermomicrobia were relatively abundant and diverse, accounting for 4.66,
221 3.75, 3.13, 2.96, 1.62 and 1.36% of the total sequences, respectively, and 3.42, 4.41, 14.76, 0.61,
222 3.97 and 1.19% of the total OTUs, respectively (Figure S2).

223 At the class level, 92 bacteria taxa were identified. The dominant 19 taxa (relative
224 abundance > 1%) accounted for >88.43% of the sequences in each sample. The most abundant
225 taxa (relative abundance > 5%) were Acidobacteria (16.19%), Alphaproteobacteria (10.11%),
226 Betaproteobacteria (9.48%), Thermoleophilia (9.22%), Actinobacteria (7.31%),
227 Deltaproteobacteria (5.77%) and Gammaproteobacteria (5.29%). The other relatively abundant
228 taxa included Gemmatimonadetes, Bacilli, Nitrospira, MB-A2-108, Planctomycetacia,

Acidimicrobiia, Anaerolineae, KD4-96, Holophagae, Thermomicrobia, Sphingobacteriia and TK10, each accounting for 1.05–4.66% of the total sequences (Figure 1A).

At the genus level, 494 taxa were detected, accounting for 31.41% of the total sequences; and the top 50 taxa accounted for 75.04% of the total identified sequences at genus level. The dominant genera (relative abundance > 1%) were *Bacillus*, *Gaiella*, *Mizugakiibacter* and *Streptomyces*, accounting for 2.22, 1.79, 1.35 and 1.01% of the total sequences, respectively. Relatively abundant genera were *Sphingomonas* (0.90%), *Haliangium* (0.89%), *Gemmatimonas* (0.80%), *Bryobacter* (0.76%), *Nocardioides* (0.74%), *Arthrobacter* (0.71%), *Steroidobacter* (0.68%), *Solirubrobacter* (0.66%) and *Microvirga* (0.57%) (Figure 1B).

Bacterial Community Variation at Different Levels with Monocropping Time

In order to illuminate the bacterial community variation across long-term monocropping time of peanut, UPGMA, PCA and PCoA were used to cluster the bacterial communities within soil samples. Overall, the UPGMA tree showed that replicate samples were grouped together, and soil samples with similar monocropping time but different peanut varieties were clustered into a group (Figure 2). Both PCA and PCoA also showed obvious clustering of bacterial communities based on monocropping time (Figure 3, Figure S3). Samples from 1- and 2-year monocropping plots were grouped into one class, and samples from 11- and 12-year plots were grouped into another. However, the cluster distance was shorter among samples from 1- and 2-year than among samples from 11- and 12-year monocropping plots. These analyses indicated that bacterial communities were significantly influenced by monocropping period of peanut, and

showed more obvious changes under long-term monocropping.

The heatmap analyses showed that soil bacterial community composition was significantly diverse at different levels between short- and long-term monocropping. At the phylum level, among the 41 bacteria taxa identified, nine taxa showed obvious abundance decrease with monocropping time and five taxa showed obvious abundance increase (Figure S4, Figure 4). The average abundance of Acidobacteria accounted for 20.30 and 19.83% in the 1- and 2-year libraries, respectively, and decreased to 15.74 and 16.28% in the 11- and 12-year libraries. The phyla Planctomycetes, Nitrospirae and Bacteroidetes were relatively abundant in the 1- and 2-year libraries, accounting for 3.12, 2.96 and 1.62% of the total sequences, respectively, and correspondingly decreased in the 11- and 12-year libraries by 20.23, 17.47 and 27.81%. Additionally, the taxa Armatimonadetes, Latescibacteria, JL-ETNP-Z39, Thermotogae and Caldiserica, for which relative abundances were <1%, also had clear decreasing tendencies. In general, decreases in their abundance were accompanied by decreases in their diversity (Table S2). In contrast, the abundance of some taxa increased with monocropping time. The average abundance of Gemmatimonadetes, Firmicutes and Elusimicrobia accounted for 3.91, 2.65 and 0.16% in the short-term monocropping libraries, respectively, and correspondingly increased to 5.40, 4.84 and 0.21% for the long-term. The other taxa with increasing abundance all had relatively low abundance, accounting for 0.01–0.04% of the total sequences, and included Parcubacteria and Chlamydiae. The diversity of the three relatively abundant taxa (Gemmatimonadetes, Firmicutes and Elusimicrobia) did not show obvious changes with monocropping time, but that of the other low-abundance taxa tended to increase with

monocropping time (Table S2).

Analysis of the microbial community at lower taxonomic level (genus or species) can provide better phylogenetic resolution than at higher taxonomic levels (*Ho et al., 2016*). At genera level, the top 50 most-abundant taxa accounted for 75.04% of the total identified sequences that were affiliated with 494 taxa. The abundance distributions of the top 50 genera in each sample were presented in a heatmap (Figure 5). Some taxa showed obvious changes with monocropping time (Figures S5 and S6). The dominant genera *Bacillus* (2.22%) and *Mizugakiibacter* (1.35%) increased their average abundance from 1.49 and 0.67%, respectively, in the 1- and 2-year libraries to 2.96 and 2.02% in the 11- and 12-year libraries. However, the number of OTUs affiliated with the genera *Bacillus* (13–14 OTUs) and *Mizugakiibacter* (four OTUs) in each sample was relatively low and changed little. Seven genera (*Gemmatimonas*, *Marmoricola*, *Candidatus* Solibacter, *Tumebacillus*, *Jatrophihabitans*, *Sporosarcina* and *Pseudolabrys*) accounted for 0.17–0.80% of the total sequences, and increased their average abundance by >90%. The genera *Nocardioides*, *Solirubrobacter* and *Reyranella* accounting for 0.21–0.74% of the total sequences also showed increased abundance with monocropping time, with increase rates of 26.44–57.20%. Some taxa showed an opposite trend, with abundance decreasing with monocropping time. They were *Steroidobacter*, *Candidatus Entotheonella*, *Nitrosospira*, *Pirellula*, *Piscinibacter*, *Ramlibacter*, *Lysobacter*, *Skermanella* and *Planctomyces*, which accounted for 0.20–0.68% of the total sequences, and the abundance decrease rate ranged within 12.86–51.13%. Overall, abundance of these genera began to decline in the 2-year monocropping libraries, and decreased more for the long-term libraries.

Most sequences, accounting for 91.54% of the total sequences, were not classified at the species level, but some taxa among the identified species also obvious changed with monocropping time (Figure S7, Figure 6). Among the top 10 most-abundant species, *Bacillus aryabhattai* and *Bacillus funiculus* accounting for 0.58 and 0.42% of the total sequences, respectively, showed increased abundance in long- compared to short-term monocropping libraries. The increase rate of these two taxa reached 116.23 and 177.70%, respectively. The relatively abundant taxa of *Bacillus luciferensis*, *Tumebacillus ginsengisoli*, *Bacillus decolorationis* and *Streptacidiphilus luteoalbus*, accounting for 0.01–0.10% of the total sequences, increased their relative abundances by >118%. The taxa *Paenibacillus alginolyticus* and *Streptosporangium violaceochromogenes* also presented increased abundance with increase rates ranging within 52.07–84.49%, but the change trends differed among peanut varieties. The species *Paenibacillus alginolyticus* and *Streptosporangium violaceochromogenes* showed no obvious abundance changes in Huayu 917 and Huayu 50 libraries, respectively. In contrast to the above taxa, *Chitinophaga ginsengihumi*, *Lysobacter yangpyeongensis* and *Phyllobacterium myrsinacearum*, accounting for 0.03–0.05% of the total sequences, showed obvious decreases in abundance and the abundance decrease rate ranged within 32.57–61.59%.

Prediction of bacteria community functions

The predominant category of the predicted functional genes was affiliated with metabolism (48.71%), followed by genetic information (21.03%), environmental information processing (12.89%) and cellular processes (7.62%) (Figure S8). The PCA was used to cluster the predicted

functional pathway within soil samples at three KEGG levels. The analyses at KEGG levels 2 and 3 and KEGG ortholog (KO) level all showed similar clusters to that of bacterial community structure (Figure 7, Figure S9). Samples from 1- and 2-year monocropping plots were grouped into one class, and samples from 11- and 12-year plots were grouped into another.

Bacterial community functions also presented significant changes at different pathway levels across long-term monocropping. For KEGG pathways at KEGG level 2 that are involved in carbohydrate metabolism, endocrine system, excretory system, nucleotide metabolism, transport and catabolism, transcription, biosynthesis of other secondary metabolites and aging showed decreasing trends with long-term monocropping, but pathways affiliated with membrane transport and cellular community prokaryotes showed increased abundance (Table S3). There were 390 KEGG pathways at KEGG level 3 predicted in our libraries. The abundant pathways (>1.0%) that presented obvious abundance changes with monocropping period were further examined. The pathways involved in DNA repair and recombination proteins, purine metabolism, transfer RNA biogenesis, exosome, amino acid related enzymes, pyrimidine metabolism, mitochondrial biogenesis, ribosome, oxidative phosphorylation, carbon fixation pathways in prokaryotes, aminoacyl tRNA biosynthesis as well as alanine, aspartate and glutamate metabolism showed decreased abundance in long- compared to short-term monocropping samples. In contrast, pathways involved in ribosome biogenesis, messenger RNA biogenesis, RNA degradation, quorum sensing, glyoxylate and dicarboxylate metabolism and lipid biosynthesis proteins were enriched in long-term monocropping samples (Table S4).

DISCUSSION

It has been demonstrated that soil microorganisms play influential roles in the productivity and sustainability of agricultural systems (*Van der Heijden and Wagg, 2013; Vukicevich et al. 2016*). 'Soil sickness' caused by continuous peanut monocropping could be closely related to the dynamics of soil microbial communities. Our previous study using library analysis showed that soil microbial community structure shifted during three years of continuous peanut monocropping (*Chen et al., 2012; Chen et al., 2014*). Li *et al*. reported that consecutive peanut monoculture changed communities of soil nematodes and fungi (*Li et al., 2014b; Li et al., 2015*). However, the specific dynamic succession of the bacterial community, which is most diverse in soil – under long-term peanut monocropping is not clear.

In the present study, we selected four peanut varieties with different monocropping responses and recorded the detailed feedback responses of their root soil bacteria communities to short- and long-term consecutive monocropping using high-throughput sequencing. Bacterial richness and diversity were measured by OTU number and Chao1 and Shannon indexes, as well as rarefaction curves. They all indicated that the majority of bacterial diversity was covered by the surveying effort and the diversity of the soil bacterial community generally declined with long-term peanut monocropping.

Our previous study showed that bacterial community structure presented significant dynamics during three years of peanut monocropping, but was less affected by peanut growth stage (*Chen et al. 2014*). *Li et al*. (*2014b*) reported that fungal communities were significantly selected by monocropping period of peanut, but also were less affected by growth stage in the

red soil region of southern China. In the present study, the UPGMA, PCA and PCoA methods were used to cluster the bacterial communities within the 48 samples from short- and long-term monocropping soil. The analyses showed that all samples regardless of variety from 1- and 2-year monocropping plots were grouped into one class, and samples from 11- and 12-year plots were grouped into another. However, the cluster distance between samples from 1- and 2-year plots was shorter than that between samples from 11- and 12-year plots. Additionally, the soil pH, the contents of available P, K in soil also presented obvious changes with long-term monocropping of peanut. These analyses indicated that soil pH, available P, K could be closely related with soil bacterial communities under long-term monocropping of peanut. Monocropping time had a strong influence on the microbial communities as well as physiochemical properties, but peanut variety and growth stage had little impact. Additionally, the soil bacterial community had significantly greater dynamics under long- compared to short-term monocropping time.

Our previous study demonstrated that bacterial communities at different taxonomic levels showed obvious dynamics during three years of peanut monocropping (*Chen et al. 2014*). Most of the obviously changed taxa at order level showed abundance and diversity declines with monocropping time and only several taxa showed increased abundance and diversity (*Chen et al. 2014*). A third cropping of Jerusalem artichoke also decreased bacterial alpha-diversity compared to 1–2 years of monocropping (*Zhou et al., 2018*). In this study, under long-term peanut monocropping, the alpha-diversity of the soil bacterial community generally decreased. At phyla level, nine taxa that accounted for 0.01–18.04% of the total sequences showed obvious decreases

372 in abundance with monocropping time and generally their decreased abundance was
373 accompanied by a decrease in diversity. Five taxa, representing 0.01–4.66% of the total
374 sequences, showed obvious increases in abundance: the three relatively abundant taxa showed no
375 obvious change in diversity with monocropping time, but that of the other two less abundant taxa
376 showed increasing trends. It was suggested that simplification of bacterial communities is a
377 common phenomenon during monocropping; however, some taxa increased their abundance and
378 diversity, possibly due to their adaptability to a new microenvironment.

379 Soil microbe modifications could contribute to peanut soil sickness (*Chen et al., 2014; Li et*
380 *al., 2014b*). Li *et al*. (*2012*) reported that bacteria proportion in total PLFA decreased from 67.4
381 to 53.0% in a peanut monocropping system, whereas the proportion of fungi increased from 16.9
382 to 32.8%. Consecutive peanut monocropping resulted in the selection of pathogenic and
383 beneficial fungi (*Chen et al., 2012; Li et al., 2014b*). Soil nematode abundance and functional
384 composition also changed with continuous peanut monocropping (*Li et al., 2015*). In our study,
385 the bacteria community compositions and predicted functions at different levels all presented
386 obvious changes with long-term peanut monocropping.

387 Some taxa that were identified at lower taxonomic levels, such as genus or species, showed
388 significant changes in abundance with long-term peanut monocropping. At genera level,
389 *Steroidobacter*, *Candidatus Entotheonella*, *Nitrosospira*, *Pirellula*, *Piscinibacter*, *Ramlibacter*,
390 *Lysobacter*, *Skermanella* and *Planctomyces* showed decreased abundance with monocropping
391 time. At the species level, *Chitinophaga ginsengihumi*, *Lysobacter yangpyeongensis* and
392 *Phyllobacterium myrsinacearum* also showed obvious decrease with long-term monocropping.

393 Several taxa found were beneficial to plant growth. The genus *Nitrosospira* is reportedly related
394 to nitrogen cycle progress (*Mellbye et al., 2017*) and *Candidatus Entotheonella* is related to
395 biosynthesis (*Uria et al., 2018*). Additionally, at the species level, *Phyllobacterium*
396 *myrsinacearum* functions in nitrogen-fixing (*Gonzalez-Bashan et al., 2000*). The study
397 concerning roles in plants of the other decreased taxa at the genus or species levels were not
398 found. However, this also demonstrated simplification of the beneficial bacteria community with
399 long-term peanut monocropping.

400 In contrast, some taxa at the genus or species levels showed increased abundance with long-
401 term peanut monocropping. The genus *Bacillus* was dominant (2.22%) in our libraries, and
402 increased their average abundance from 1.49% in 1- and 2-year to 2.96% in the 11- and 12-year
403 libraries. However, the number of OTUs affiliated with the *Bacillus* genus in each sample (13–14
404 OTUs) was relatively low and changed little. This may suggest that identified members of
405 *Bacillus* had good adaptability to the soil environment under long-term peanut monocropping.
406 *Bacillus* species are reported as the most common biocontrol agents and have important traits
407 such as plant growth-promoting properties (*Santoyo et al., 2012; Gomaa et al., 2012*). The
408 dominant species *Bacillus aryabhattai* and *Bacillus funiculus* accounting for 0.58% and 0.42% of
409 the total sequences, respectively, and the relatively abundant *Bacillus luciferensis* and *Bacillus*
410 *decolorationis* accounting for 0.10 and 0.02% of the total sequences, respectively, all showed
411 increased abundance with long-term monocropping. It was reported that *Bacillus aryabhattai*
412 could improve growth of soybean, wheat and *Xanthium italicum*, and could also improve
413 mobilization and biofortification of zinc (*Lee et al., 2012; Ramesh et al., 2014*). *Bacillus*

414 *funiculus* is reportedly related to the degradation of sodium dodecyl sulfate (*Ajithkumar et al.,*
415 *2003*). There are no reports concerning roles in plants of *Bacillus luciferensis* and *Bacillus*
416 *decolorationis*. Additionally, several relatively abundant species, including *Tumebacillus*
417 *ginsengisoli*, *Streptacidiphilus luteoalbus*, *Paenibacillus alginolyticus* and *Streptosporangium*
418 *violaceochromogenes* also showed increased abundance with long-term peanut monocropping.
419 However, no function-related studies of them were found. At the genus level, the functions of
420 most of the increased taxa are unknown, but some studies claimed that *Nocardioides* species had
421 roles in degradation of deoxynivalenol, 2,4-dinitroanisole and melamine and its hydroxy
422 derivatives (*Ikunaga et al., 2011; Takagi et al., 2012; Fida et al., 2014*). It was reported that
423 allelochemicals from root exudates or decomposants of crops could induce autotoxicity and were
424 closely related to soil sickness (*Asaduzzaman et al., 2012; Huang et al., 2013*). These
425 allelochemicals accumulated with monocropping period, but not to high levels (*Yang et al., 2015;*
426 *Li et al., 2014a*), possibly due to interactions between allelochemicals and soil microbes (*Li et al.,*
427 *2014a; Wang et al., 2019*). Based on the analyses, the increased bacterial taxa that could promote
428 plant growth or degrade potential soil allelochemicals should be closely related to soil
429 remediation and may have potential application to relieve soil sickness under peanut
430 monocropping.

431 Function prediction analyses showed significant changes in bacterial community functions
432 at different pathway levels with long-term peanut monocropping. The analyses at KEGG levels 2
433 and 3 and KO level all showed similar clustering to that of bacterial community structure.
434 Samples from short-term monocropping plots were grouped into one class, and samples from

long-term plots were grouped into another. Comparing analyses of the abundance indicated that many detected KEGG pathways at KEGG levels 2 or 3 had obvious changes with long-term monocropping. Most had a decreasing trend with long-term peanut monocropping, and only a few showed abundance increases. Combined with the community structure variation analyses, both the bacterial community structure and function presented significant changes with long-term monocropping, and their simplification could be the main cause of soil sickness.

In conclusion, through tracking the detailed feedback responses of soil bacteria to long-term monocropping of four different peanut varieties, we provided field-based evidence that long-term monocropping could result in a general loss in bacterial diversity and remarkable changes in bacterial abundance and compositions as well as functions. Combined with our previous study (*Chen et al., 2014*), analyses in this study suggested that bacterial communities were obviously influenced by the monocropping period, but less influenced by peanut variety and growth stage. Additionally, soil pH, available P, K could be closely related with soil bacterial communities under long-term monocropping of peanut. Some bacterial taxa, with increased abundance have functions of promoting plant growth or degrading potential soil allelochemicals, should be closely related to soil remediation and may have potential application to relieve peanut soil sickness. Simplification of bacterial communities, especially beneficial communities, and bacterial community function simplification with long-term peanut monocropping could be the main cause of peanut soil sickness. In the future, we will investigate dynamics of functional genes with long-term peanut monocropping using metagenomics. These studies will improve our understanding of the mechanism underlying peanut soil sickness.

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592

Figure 1

Overall abundance distribution of bacteria at the (A) class and (B) genus levels from soil that was monocropped with peanut.

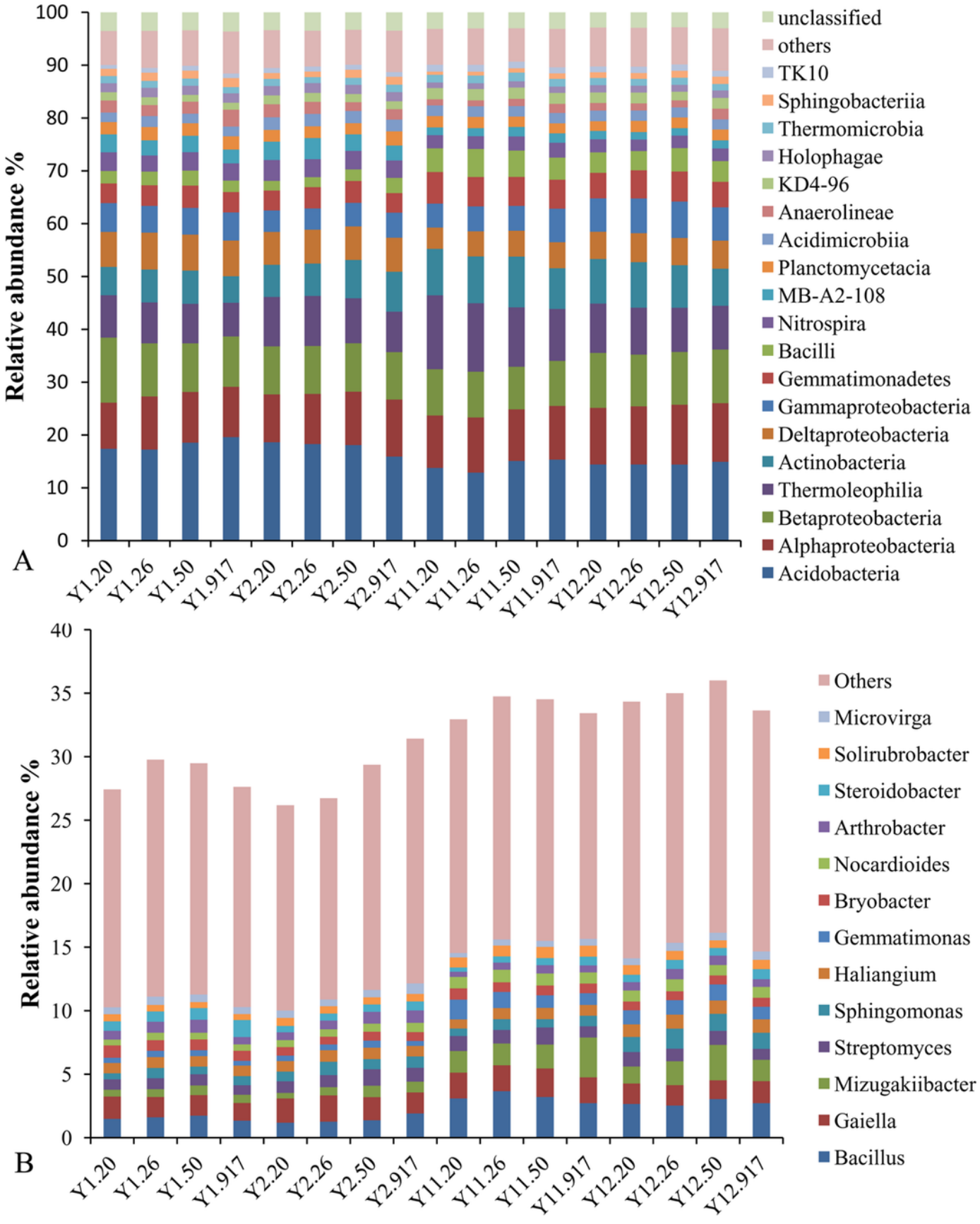
A
Relative abundance %
100
90
80
70
60
50
40
30
20
10
0
Y1.20
Y1.26
Y1.50
Y1.917
Y2.20
Y2.26
Y2.50
Y2.917
Y11.20
Y11.26
Y11.50
Y11.917
Y12.20
Y12.26
Y12.50
Y12.917
unclassified
others
TK10
Sphingobacteriia
Thermomicrobia
Holophagae
KD4-96
Anaerolineae
Acidimicrobiia
Planctomycetacia
MB-A2-108
Nitrospira
Bacilli
Gemmatimonadetes
Gammaproteobacteria
Deltaproteobacteria
Actinobacteria
Thermoleophilia
Betaproteobacteria
Alphaproteobacteria
Acidobacteria
B
Relative abundance %
40
35
30
25
20
15
10
5
0
Y1.20
Y1.26
Y1.50
Y1.917
Y2.20
Y2.26
Y2.50
Y2.917
Y11.20
Y11.26
Y11.50
Y11.917
Y12.20
Y12.26
Y12.50
Y12.917
Others
Microvirga
Solirubrobacter
Steroidobacter
Arthrobacter
Nocardioides
Bryobacter
Gemmatimonas
Haliangium
Sphingomonas
Streptomyces
Mizugakiibacter
Gaiella
Bacillus

Figure 2

The UPGMA tree showing the clusters for bacterial communities in short- and long-term monocropped soils based on the unweighted UniFrac value.

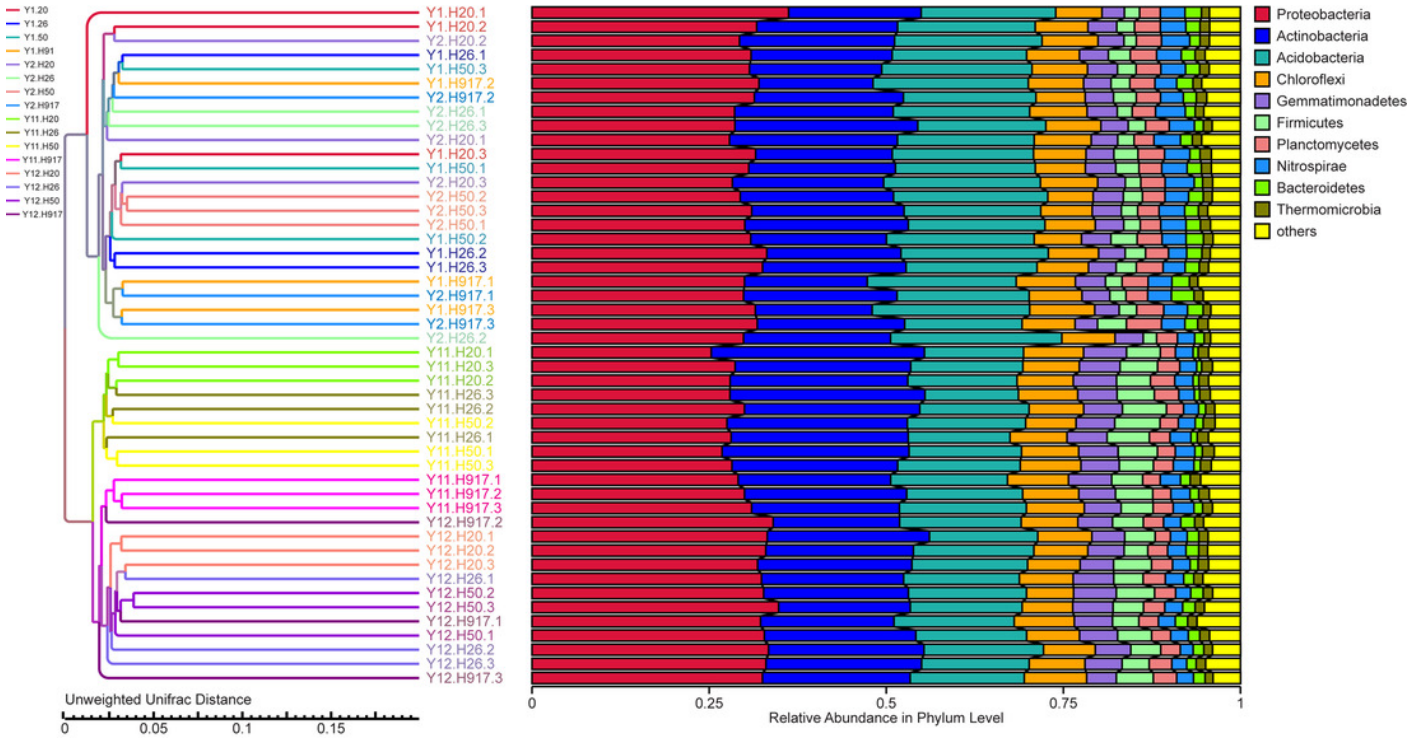

Figure 3

Principal component analysis (PCA) of bacterial distributions in short- and long-term monocropped peanut soils for different peanut varieties.

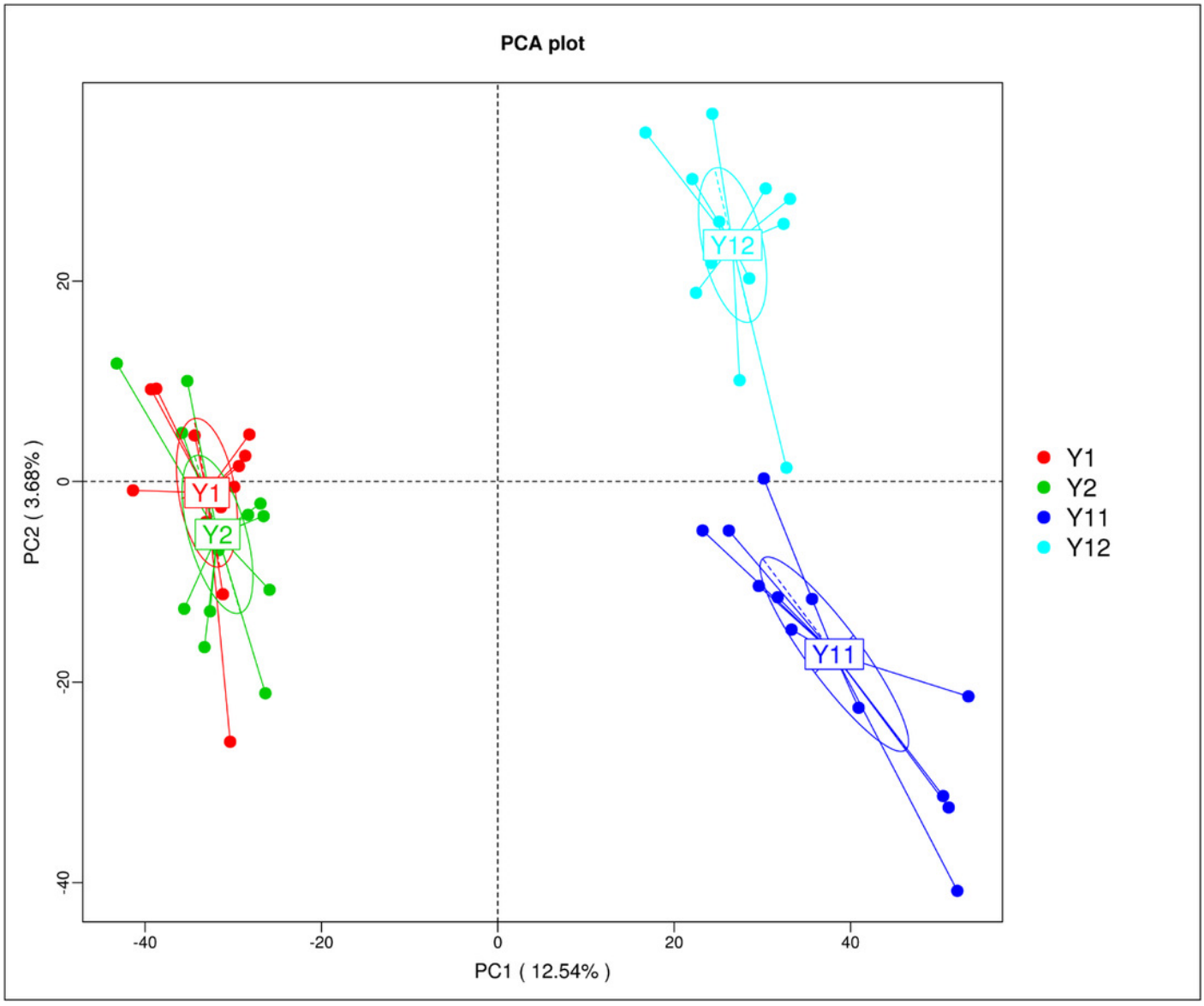

Figure 4

Relative abundance of significantly changed taxa at phylum level in plots that were monocropped with peanuts for different periods.

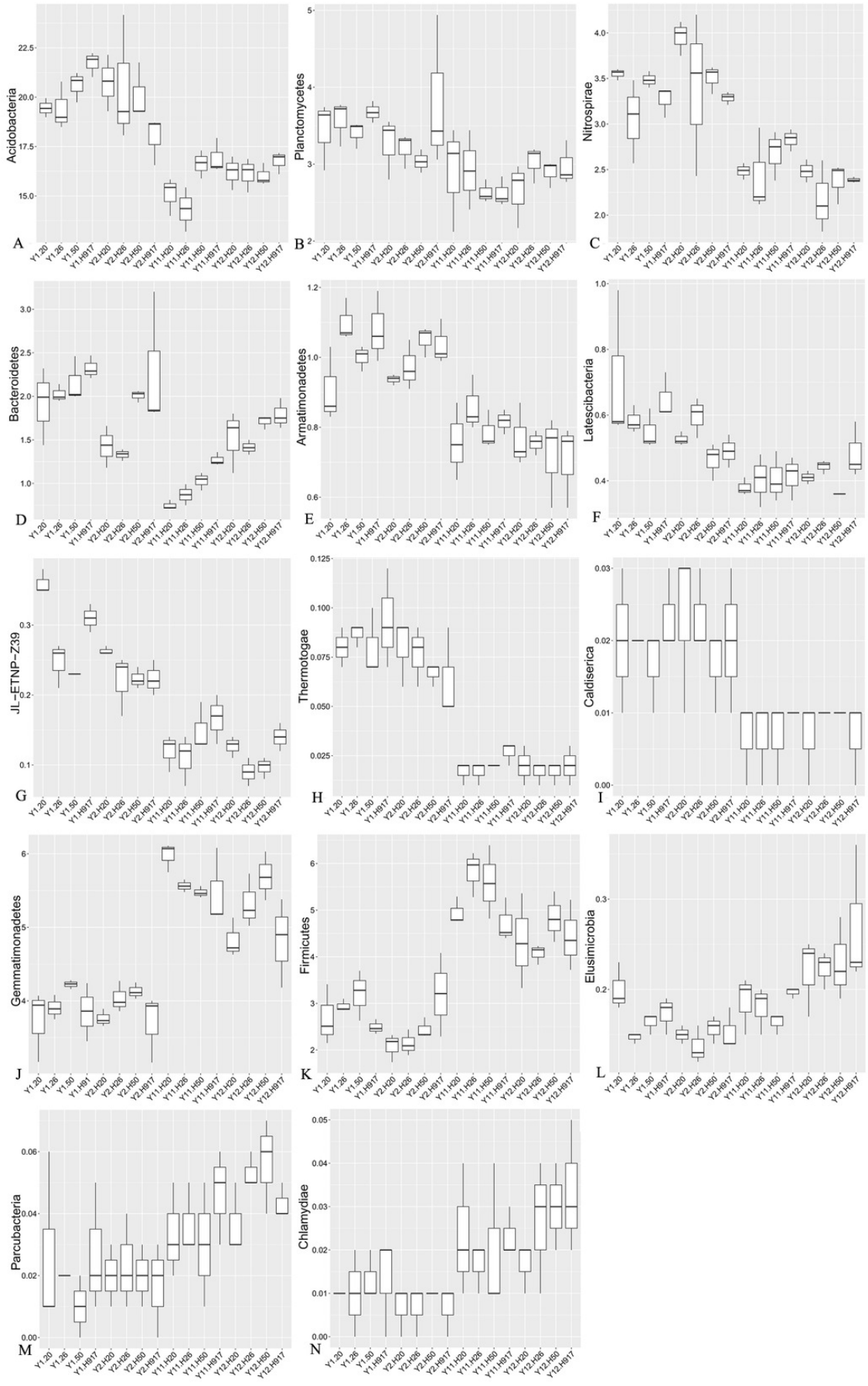
A
Acidobacteria
B
Planctomycetes
C
Nitrospirae
D
Bacteroidetes
E
Armatimonadetes
F
Latescibacteria
G
JL-ETNP-Z39
H
Thermotogae
I
Caldiserica
J
Gemmatimonadetes
K
Firmicutes
L
Elusimicrobia
M
Parcubacteria
N
Chlamydiae
Y1.20 Y1.26 Y1.50 Y1.H917 Y2.H20 Y2.H26 Y2.H50 Y2.H917 Y11.H20 Y11.H26 Y11.H50 Y11.H917 Y12.H20 Y12.H26 Y12.H50 Y12.H917

Figure 5

Heatmap presenting the distribution of the top 50 taxa at genus level in 16 monocropped peanut plots.

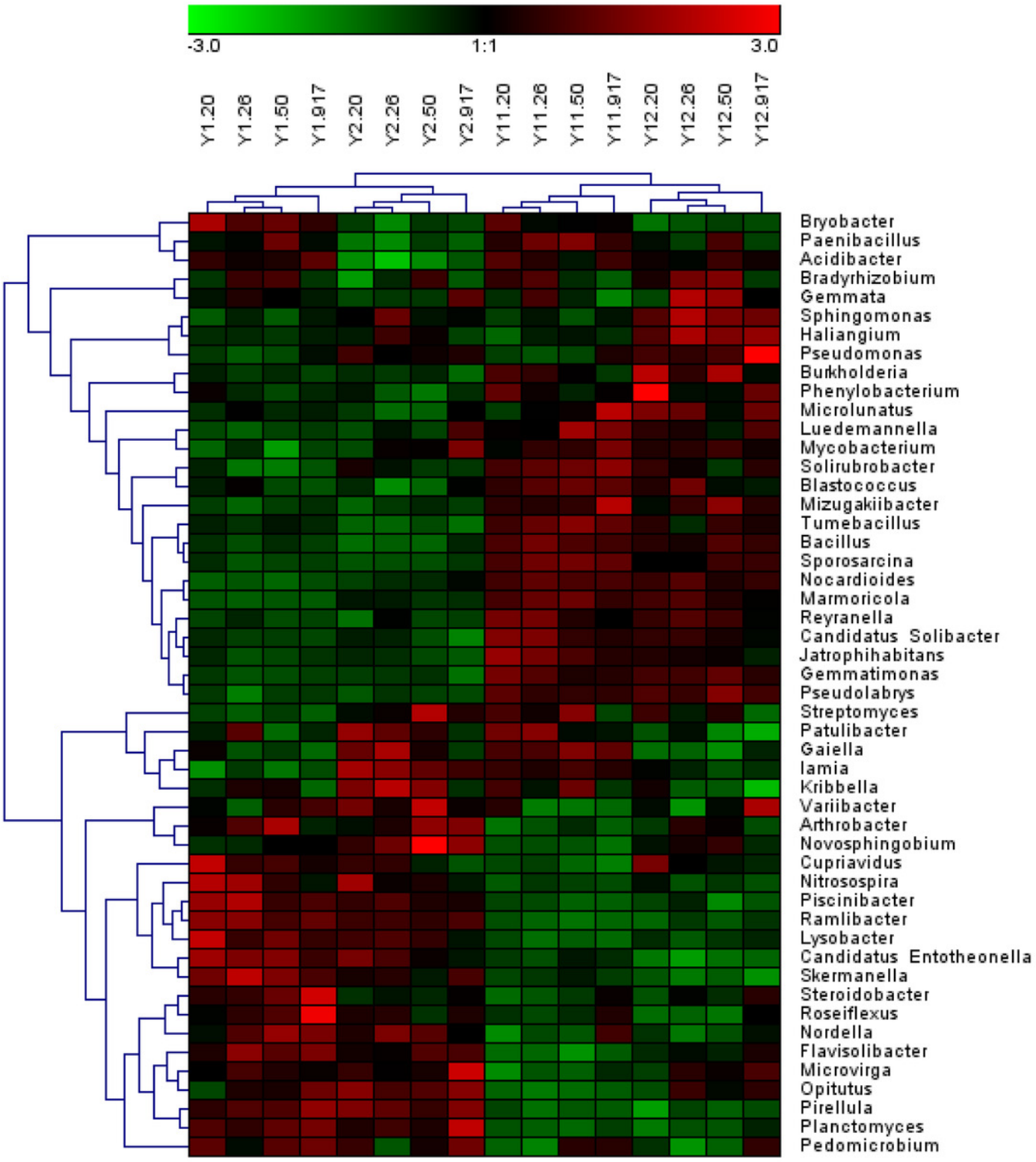
-3.0
1:1
3.0
Y1.20
Y1.26
Y1.50
Y1.917
Y2.20
Y2.26
Y2.50
Y2.917
Y11.20
Y11.26
Y11.50
Y11.917
Y12.20
Y12.26
Y12.50
Y12.917
Bryobacter
Paenibacillus
Acidibacter
Bradyrhizobium
Gemmata
Sphingomonas
Haliangium
Pseudomonas
Burkholderia
Phenylobacterium
Microlunatus
Luedemannella
Mycobacterium
Solirubrobacter
Blastococcus
Mizugakiibacter
Tumebacillus
Bacillus
Sporosarcina
Nocardioides
Marmoricola
Reyranella
Candidatus Solibacter
Jatrophihabitans
Gemmatimonas
Pseudolabrys
Streptomyces
Patulibacter
Gaiella
Iamia
Kribbella
Variibacter
Arthrobacter
Novosphingobium
Cupriavidus
Nitrosospira
Piscinibacter
Ramlibacter
Lysobacter
Candidatus Entotheonella
Skermanella
Steroidobacter
Roseiflexus
Nordella
Flavisolibacter
Microvirga
Opitutus
Pirellula
Planctomyces
Pedomicrobium

Figure 6

Relative abundance of significantly changed taxa at species level in plots that were monocropped with peanuts for different periods.

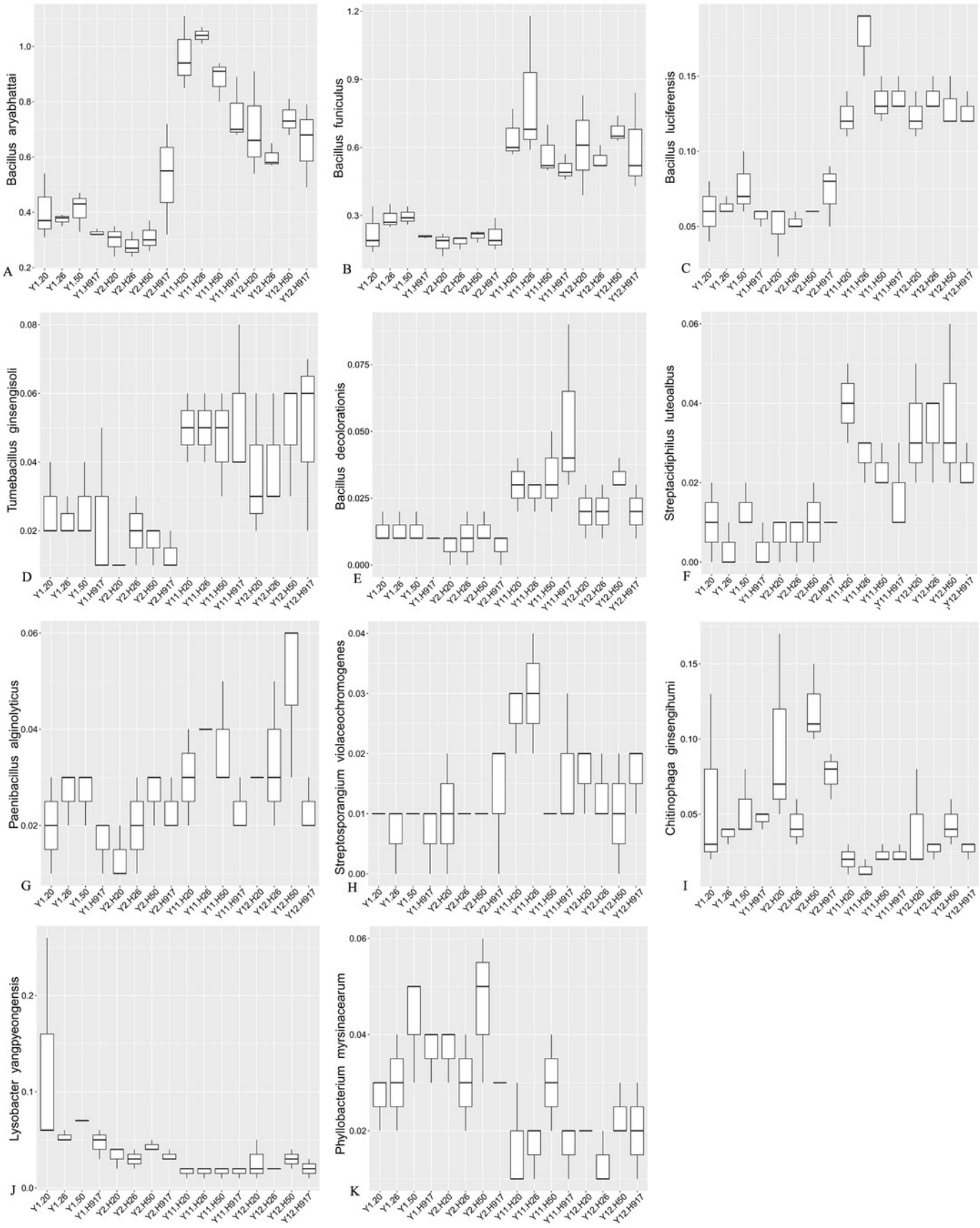
A
Bacillus aryabhattai
B
Bacillus funiculus
C
Bacillus luciferensis
D
Tumebacillus ginsengisoli
E
Bacillus decolorationis
F
Streptacidiphilus luteoalbus
G
Paenibacillus alginolyticus
H
Streptosporangium violaceochromogenes
I
Chitinophaga ginsengihumi
J
Lysobacter yangpyeongensis
K
Phyllobacterium myrsinacearum
Y1.20 Y1.26 Y1.50 Y1.H917 Y2.H20 Y2.H26 Y2.H50 Y2.H917 Y11.H20 Y11.H26 Y11.H50 Y11.H917 Y12.H20 Y12.H26 Y12.H50 Y12.H917

Figure 7

Principal component analysis (PCA) of predicted bacterial functions at KEGG level 2 in short- and long-term monocropped peanut soils for different peanut varieties.

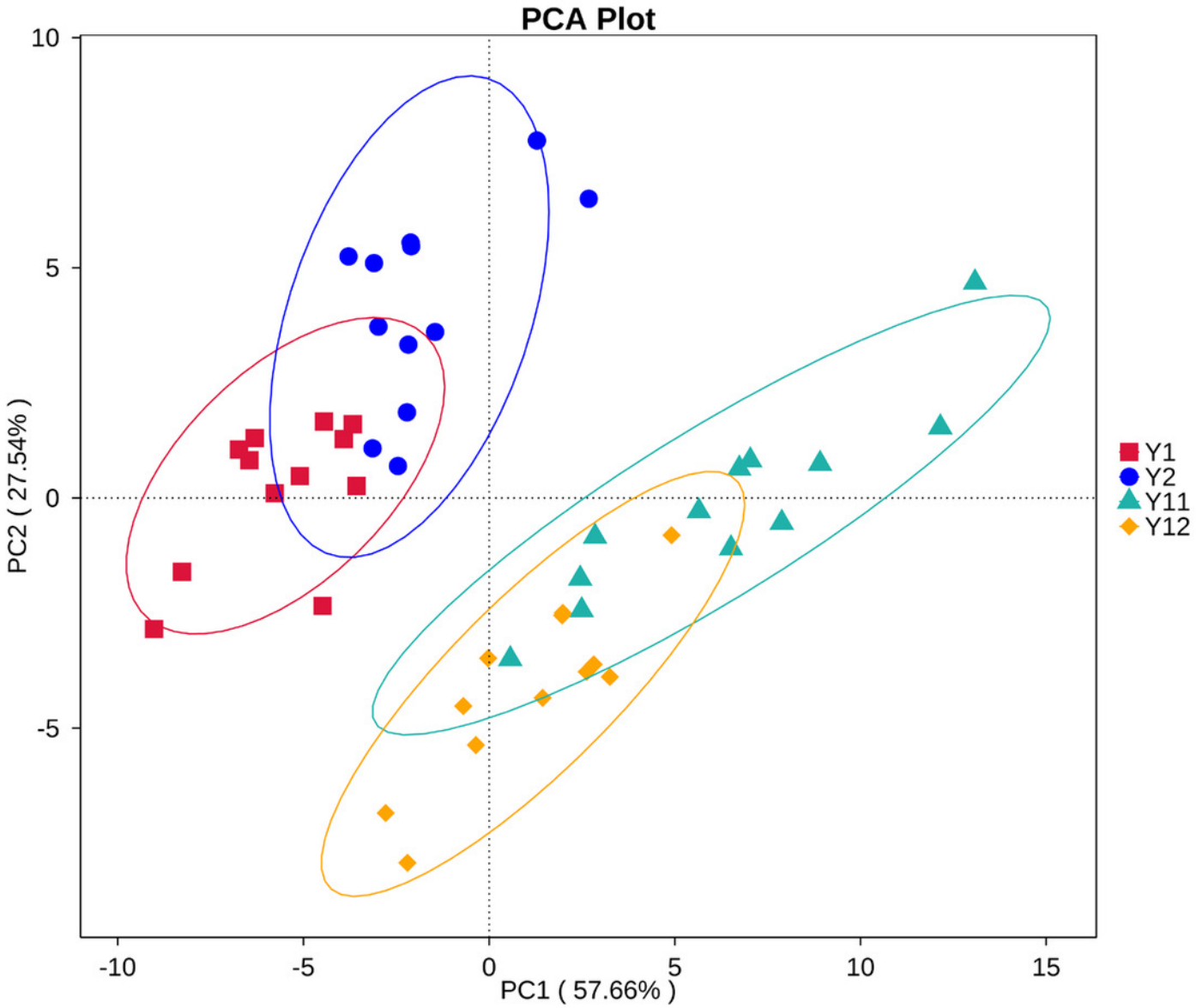

Table 1(on next page)

Overview of soil samples obtained from plots that were monocropped for different periods.

The table shows the number of quality sequences and the indexes for α-diversity.

Operational taxonomic units (OTUs) are defined at 97% sequence similarity.

Table 1. Overview of soil samples obtained from plots that were monocropped for different periods. The table shows the number of quality sequences and the indexes for α-diversity. Operational taxonomic units (OTUs) are defined at 97% sequence similarity.

Sample name	Monocropping years	peanut varieties	Sequenced library No.	Total filtered quality sequences	Richness estimates		Diversity estimates
					OTUs	Chao1	Shannon
Y1.H20	1	Huayu 20	3	211427	5132±327	4296±727	9.92±0.22
Y1.H26	1	Huayu 26	3	201027	5262±105	4287±118	10.10±0.06
Y1.H50	1	Huayu 50	3	217300	5187±66	4189±86	10.02±0.04
Y1.H917	1	Huayu 917	3	209336	5212±33	4439±432	10.01±0.05
Y2.H20	2	Huayu 20	3	207920	5074±69	4303±444	9.93±0.04
Y2.H26	2	Huayu 26	3	197796	5083±139	4075±166	9.93±0.11
Y2.H50	2	Huayu 50	3	220390	5118±23	4445±274	9.99±0.03
Y2.H917	2	Huayu 917	3	227617	5462±176	4839±517	10.14±0.08
Y11.H20	11	Huayu 20	3	219846	4619±181	3756±212	9.70±0.15
Y11.H26	11	Huayu 26	3	196398	4778±71	3852±115	9.83±0.06
Y11.H50	11	Huayu 50	3	196570	4693±39	3742±51	9.83±0.00
Y11.H917	11	Huayu 917	3	229162	5064±67	4428±369	9.90±0.05
Y12.H20	12	Huayu 20	3	210578	5036±62	4056±68	9.93±0.05
Y12.H26	12	Huayu 26	3	212672	5120±56	4294±363	9.96±0.02
Y12.H50	12	Huayu 50	3	199615	4971±63	4209±321	9.91±0.07
Y12.H917	12	Huayu 917	3	197203	5049±233	4017±328	9.97±0.07