

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. 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. 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.

KEY WORDS

Long-term monocropping, *Arachis hypogaea* L., soil sickness, soil bacterial community, sustainable agroecosystem

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

Peanut, an important oil and economic crop worldwide, is very adaptable to climatic conditions and grows in tropical, subtropical and warm temperate climate regions across the world. Due to limitations of arable land and requirements for developing regional agro-industrialization, large-scale monocropping of peanut is common in China (*Chen et al., 2016*). Research indicates that consecutive peanut monocropping has caused a decline in yield and quality and increases in disease pressures (*Wang et al., 2005*). Early studies showed that soil

diversity and abundance of bacterial communities changed with continuous peanut monocropping according to phospholipid fatty acid (PLFA), denaturing gradient gel electrophoresis and library analyses (*Li et al., 2012; Chen et al., 2014; Liu et al., 2015*). Our earlier study also indicated that the balance of soil bacterial communities was disturbed during three years of continuous monocropping (*Chen et al., 2014*). However, the specific characteristics of the soil bacterial community and the changes of soil bacterial structure and composition in response to long-term peanut monocropping are unclear.

In this study, based on a gradient that we previously constructed of continuously monocropping in a peanut field, we analyzed and compared responses of root soil bacterial communities of four peanut varieties to monocropping for 1, 2, 11 and 12 years, using high-throughput sequencing techniques. This is the first study to investigate bacterial community dynamics succession under long-term peanut monocropping based on monocropping gradient experiment plots with a consistent background. The aims of this study were to (i) determine the change characteristics of the soil bacterial community and the influences of peanut varieties on the dynamics of the bacterial community, under long-term continuous monocropping of peanut and (ii) identify the key bacteria taxa related to peanut soil sickness.

MATERIALS AND METHODS

Field experiment and soil sampling

The field experimental site was set up in Laixi experimental farm at the Shandong Peanut Research Institute, Qingdao, China (36° 50' N, 120° 31' E). A gradient of consecutive

monocropped peanut experiment plots that we previously developed was used. Independent pools were applied, with the size of each pool being 4 m long, 1.5 m wide and 1 m high. We collected soil from the plow layer of the cultivated land, thoroughly mixed and then added to the pools. Peanuts were planted in May each year and harvested in October, all field management, including the planting pattern and the use of water and fertilizer were consistent among different pools. In addition, to minimize the influence of other factors including variety on the measured indexes, the planting position of the same peanut variety was fixed during the continuous cropping period. The longest-running pools have now been continuously monocropped with peanuts for 16 years.

We selected four peanut varieties with distinct responses to monocropping to analyze their soil bacterial community structure in plots monocropped for 1, 2, 11 and 12 years. The four varieties included two large fruit varieties, Huayu 917 and Huayu 50, and two small fruit varieties, Huayu 26 and Huayu 20. Based on previous testing of their yield-related indexes, Huayu 20 was the most sensitive to long-term monocropping, Huayu 917 was intermediate and Huayu 26 and Huayu 50 were tolerant. The soil sampling method followed the protocol of Chen *et al.* (2014) and three repeats were collected for each soil sample at full-bloom stage.

DNA extraction, pcr and high-throughput sequencing

For each soil sample, total DNA was extracted with the Power Soil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) following the manufacturer's instructions. The

concentration and purity of the DNA was checked by electrophoresis on 1.0 % (w/v) agarose 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™ 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

Characteristics of sequencing data

In order to compare the soil bacterial community structure and composition of the four

peanut varieties to short- and long-term monocropping periods, a total of 48 16S rRNA gene libraries were analyzed using high-throughput sequencing technique. The sampling variables, including four peanut varieties and four monocropping periods, are shown in Table 1. A total of 2,541,421 quality-filtered sequences obtained from the 48 samples, ranging within 37,605–68,117 with an average length of 373 bp, resulted in identification of a total of 9028 OTUs applying a 3% sequence dissimilarity cutoff. After data normalization, 1,805,040 quality-filtered sequences affiliated with 8856 OTUs were obtained. The bacterial complexity of the 48 samples was estimated on the basis of alpha-diversity (OTU number, Chao1 index and Shannon index) and showed relatively higher bacterial diversity in samples from the 1- and 2-year compared to the 11- and 12-year monocropped plots (Table 1). Rarefaction curves (Figure S1) and indices of richness and diversity including all samples' Chao1 and Shannon indexes (Table 1) tended to approach a saturation plateau, indicating that the majority of bacterial diversity was recovered by the surveying effort.

Bacterial community structure and composition

In total, 99.73% of the identified 8856 OTUs were related to bacteria, and the other 24 OTUs accounting for 0.15% of the total quality-filtered sequences were affiliated with Archaea. There were 41 bacteria phyla detected across all samples and 0.73% of bacterial sequences were unclassified at phylum level. The top 10 phyla accounted for 95.49% of the total sequences and 79.67% of the total OTUs. The dominant phyla across all samples were Proteobacteria, Actinobacteria, Acidobacteria and Chloroflexi, accounting for 30.71, 21.59, 18.04 and 7.69% of

the total sequences, respectively, and 26.61, 7.43, 6.49 and 10.77% of the total 8856 OTUs, respectively. The phyla Gemmatimonadetes, Firmicutes, Planctomycetes, Nitrospirae, Bacteroidetes and Thermomicrobia were relatively abundant and diverse, accounting for 4.66, 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, 3.97 and 1.19% of the total OTUs, respectively (Figure S2).

At the class level, 92 bacteria taxa were identified. The dominant 19 taxa (relative abundance > 1%) accounted for >88.43% of the sequences in each sample. The most abundant taxa (relative abundance > 5%) were Acidobacteria (16.19%), Alphaproteobacteria (10.11%), Betaproteobacteria (9.48%), Thermoleophilia (9.22%), Actinobacteria (7.31%), Deltaproteobacteria (5.77%) and Gammaproteobacteria (5.29%). The other relatively abundant 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 S1). 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 S1).

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*,

246 *Marmoricola*, *Candidatus Solibacter*, *Tumebacillus*, *Jatrophihabitans*, *Sporosarcina* and
 247 *Pseudolabrys*) accounted for 0.17–0.80% of the total sequences, and increased their average
 248 abundance by >90%. The genera *Nocardioides*, *Solirubrobacter* and *Reyranella* accounting for
 249 0.21–0.74% of the total sequences also showed increased abundance with monocropping time,
 250 with increase rates of 26.44–57.20%. Some taxa showed an opposite trend, with abundance
 251 decreasing with monocropping time. They were *Steroidobacter*, *Candidatus Entotheonella*,
 252 *Nitrosospira*, *Pirellula*, *Piscinibacter*, *Ramlibacter*, *Lysobacter*, *Skermanella* and *Planctomyces*,
 253 which accounted for 0.20–0.68% of the total sequences, and the abundance decrease rate ranged
 254 within 12.86–51.13%. Overall, abundance of these genera began to decline in the 2-year
 255 monocropping libraries, and decreased more for the long-term libraries.

256 Most sequences, accounting for 91.54% of the total sequences, were not classified at the
 257 species level, but some taxa among the identified species also obvious changed with
 258 monocropping time (Figure S7, Figure 6). Among the top 10 most-abundant species, *Bacillus*
 259 *aryabhattai* and *Bacillus funiculus* accounting for 0.58 and 0.42% of the total sequences,
 260 respectively, showed increased abundance in long- compared to short-term monocropping
 261 libraries. The increase rate of these two taxa reached 116.23 and 177.70%, respectively. The
 262 relatively abundant taxa of *Bacillus luciferensis*, *Tumebacillus ginsengisoli*, *Bacillus*
 263 *decolorationis* and *Streptacidiphilus luteoalbus*, accounting for 0.01–0.10% of the total
 264 sequences, increased their relative abundances by >118%. The taxa *Paenibacillus alginolyticus*
 265 and *Streptosporangium violaceochromogenes* also presented increased abundance with increase
 266 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 S2). 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 S3).

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

Soil microbe modifications could contribute to peanut soil sickness (*Chen et al., 2014; Li et al., 2014b*). *Li et al. (2012)* reported that bacteria proportion in total PLFA decreased from 67.4 to 53.0% in a peanut monocropping system, whereas the proportion of fungi increased from 16.9 to 32.8%. Consecutive peanut monocropping resulted in the selection of pathogenic and beneficial fungi (*Chen et al., 2012; Li et al., 2014b*). Soil nematode abundance and functional composition also changed with continuous peanut monocropping (*Li et al., 2015*). In our study,

the bacteria community compositions and predicted functions at different levels all presented obvious changes with long-term peanut monocropping.

Some taxa that were identified at lower taxonomic levels, such as genus or species, showed significant changes in abundance with long-term peanut monocropping. At genera level, *Steroidobacter*, *Candidatus Entothaeonella*, *Nitrosospora*, *Pirellula*, *Piscinibacter*, *Ramlibacter*, *Lysobacter*, *Skermanella* and *Planctomyces* showed decreased abundance with monocropping time. At the species level, *Chitinophaga ginsengihumi*, *Lysobacter yangpyeongensis* and *Phyllobacterium myrsinacearum* showed obvious change with long-term monocropping. Several taxa found were beneficial to plant growth. The genus *Nitrosospora* is reportedly related to nitrogen cycle progress (Mellbye et al., 2017) and *Candidatus Entothaeonella* is related to biosynthesis (Uria et al., 2018). Additionally, at the species level, *Phyllobacterium myrsinacearum* functions in nitrogen-fixing (Gonzalez-Bashan et al., 2000). The study concerning roles in plants of the other decreased taxa at the genus or species levels were not found. However, this also demonstrated simplification of the beneficial bacteria community with long-term peanut monocropping.

In contrast, some taxa at the genus or species levels showed increased abundance with long-term peanut monocropping. The genus *Bacillus* was dominant (2.22%) in our libraries, and increased their average abundance from 1.49% in 1- and 2-year to 2.96% in the 11- and 12-year libraries. However, the number of OTUs affiliated with the *Bacillus* genus in each sample (13–14 OTUs) was relatively low and changed little. This may suggest that identified members of *Bacillus* had good adaptability to the soil environment under long-term peanut monocropping.

368 *Bacillus* species are reported as the most common biocontrol agents and have important traits
 369 such as plant growth-promoting properties (Santoyo *et al.*, 2012; Gomaa *et al.*, 2012). The
 370 dominant species *Bacillus aryabhattai* and *Bacillus funiculus* accounting for 0.58% and 0.42% of
 371 the total sequences, respectively, and the relatively abundant *Bacillus luciferensis* and *Bacillus*
 372 *decolorationis* accounting for 0.10 and 0.02% of the total sequences, respectively, all showed
 373 increased abundance with long-term monocropping. It was reported that *Bacillus aryabhattai*
 374 could improve growth of soybean, wheat and *Xanthium italicum*, and could also improve
 375 mobilization and biofortification of zinc (Lee *et al.*, 2012; Ramesh *et al.*, 2014). *Bacillus*
 376 *funiculus* is reportedly related to the degradation of sodium dodecyl sulfate (Ajithkumar *et al.*,
 377 2003). There are no reports concerning roles in plants of *Bacillus luciferensis* and *Bacillus*
 378 *decolorationis*. Additionally, several relatively abundant species, including *Tumebacillus*
 379 *ginsengisoli*, *Streptacidiphilus luteoalbus*, *Paenibacillus alginolyticus* and *Streptosporangium*
 380 *violaceochromogenes* also showed increased abundance with long-term peanut monocropping.
 381 However, no function-related studies of them were found. At the genus level, the functions of
 382 most of the increased taxa are unknown, but some studies claimed that *Nocardioides* species had
 383 roles in degradation of deoxynivalenol, 2,4-dinitroanisole and melamine and its hydroxy
 384 derivatives (Ikunaga *et al.*, 2011; Takagi *et al.*, 2012; Fida *et al.*, 2014). It was reported that
 385 allelochemicals from root exudates or decomposants of crops could induce autotoxicity and were
 386 closely related to soil sickness (Asaduzzaman *et al.*, 2012; Huang *et al.*, 2013). These
 387 allelochemicals accumulated with monocropping period, but not to high levels (Yang *et al.*, 2015;
 388 Li *et al.*, 2014a), possibly due to interactions between allelochemicals and soil microbes (Li *et al.*,

2014a; Wang *et al.*, 2019). Based on the analyses, the increased bacterial taxa that could promote plant growth or degrade potential soil allelochemicals should be closely related to soil remediation and may have potential application to relieve soil sickness under peanut monocropping.

Function prediction analyses showed significant changes in bacterial community functions at different pathway levels with long-term peanut monocropping. The analyses at KEGG levels 2 and 3 and KO level all showed similar clustering to that of bacterial community structure. 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. 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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REFERENCES

Ajithkumar VP, Ajithkumar B, Iriye R, Takamizawa, K. 2003. Degradation of sodium dodecyl sulfate by *Bacillus funiculus* NAF001 isolated from Activated Sludge. *Japanese Journal of Water Treatment*

- 431 *Biology* **39(3)**:153-156 DOI org/10.2521/jswtb.39.153.
- 432 **Asaduzzaman M, Asao T. 2012.** Autotoxicity in beans and their allelochemicals. *Scientia Horticulturae* **134**:
- 433 26-31 DOI org/10.1016/j.scienta.2011.11.035.
- 434 **Bever JD, Platt TG, Morton ER. 2012.** Microbial population and community dynamics on plant roots and
- 435 their feedbacks on plant communities. *Annual Review of Microbiology* **66**:265–283 DOI
- 436 10.1146/annurev-micro-092611-150107.
- 437 **Blagodatskaya E, Kuzyakov Y. 2013.** Active microorganisms in soil: critical review of estimation criteria
- 438 and approaches. *Soil Biology and Biochemistry* **67**:192-211 DOI org/10.1016/j.soilbio.2013.08.024.
- 439 **Buttimer C, McAuliffe O, Ross RP, Hill C, O’Mahony J, Coffey A. 2017.** Bacteriophages and bacterial
- 440 plant diseases. *Frontiers in Microbiology* **8**: 34 DOI org/10.3389/fmicb.2017.00034.
- 441 **Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG,**
- 442 **Goodrich JK, Gordon JI, Huttley GA, Kelley ST.....Knight R. 2010.** QIIME allows analysis of high-
- 443 throughput community sequencing data. *Nature Methods* **7(5)**:335-336 DOI 10.1038/nmeth.f.303.
- 444 **Chaparro JM, Sheflin AM, Manter DK, Vivanco JM. 2012.** Manipulating the soil microbiome to increase
- 445 soil health and plant fertility. *Biology and Fertility of Soils* **48(5)**:489-499 DOI org/10.1007/s00374-012-
- 446 0691-4.
- 447 **Chen M, Chi X, Pan L, Chen N, Wang T, Wang M, Yang Z, Yu S. 2016.** Research progress of soil
- 448 microenvironment and peanut continuous cropping obstacle mechanism. *Journal Anhui Agricultural*
- 449 *Science* **44(33)**:33-35 DOI 10.13989/j.cnki.0517-6611.
- 450 **Chen M, Li X, Yang Q, Chi X, Pan L, Chen N, Yang Z, Wang T, Wang M, Yu S. 2012.** Soil eukaryotic
- 451 microorganism succession as affected by continuous cropping of peanut-pathogenic and beneficial fungi
- 452 were selected. *Plos One* **7(7)**:e40659 DOI 10.1371/journal.pone.0040659.

- Chen M, Li X, Yang Q, Chi X, Pan L, Chen N, Yang Z, Wang T, Wang M, Yu S. 2014. Dynamic succession of soil bacterial community during continuous cropping of peanut (*Arachis hypogaea* L.). *Plos One* **9**(7): e101355 DOI 10.1371/journal.pone.0101355.
- Compant S, Clément C, Sessitsch A. 2010. Plant growth-promoting bacteria in the rhizo-and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry* **42**(5):669-678 DOI org/10.1016/j.soilbio.2009.11.024.
- Cook RJ. 2006. Toward cropping systems that enhance productivity and sustainability. *Proceedings of the National Academy of Sciences* **103**:18389-18394 DOI 10.1073/pnas. 0605946103
- Doornbos RF, van Loon LC, Bakker PA. 2012. Impact of root exudates and plant defense signaling on bacterial communities in the rhizosphere. A review. *Agronomy for Sustainable Development* **32**(1):227-243 DOI org/10.1007/s13593-011-0028-y.
- Edgar RC. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods* **10**(10):996-998 DOI 10.1038/nmeth.2604.
- Fida TT, Palamuru S, Pandey G, Spain JC. 2014. Aerobic biodegradation of 2, 4-dinitroanisole by *Nocardioides* sp. strain JS1661. *Applied and Environmental Microbiology* **80**(24):7725-7731 DOI 10.1128/AEM.02752-14.
- Gentry LF, Ruffo ML, Below FE. 2013. Identifying factors controlling the continuous corn yield penalty. *Agronomy Journal* **105**(2):295-303 DOI 10.2134/agronj2012.0246.
- Gomaa EZ. 2012. Chitinase production by *Bacillus thuringiensis* and *Bacillus licheniformis*: their potential in antifungal biocontrol. *The Journal of Microbiology* **50**(1):103-111 DOI org/10.1007/s12275-012-1343-y.
- Gonzalez-Bashan LE, Lebsky VK, Hernandez JP, Bustillos JJ, Bashan Y. 2000. Changes in the metabolism of the microalga *Chlorella vulgaris* when coimmobilized in alginate with the nitrogen-fixing *Phyllobacterium myrsinacearum*. *Canadian journal of microbiology* **46**(7):653-659 DOI org/10.1139/w00-041.

- 477 **Ho A, Angel R, Veraart AJ, Daebeler A, Jia Z, Kim SY, Kerckhof FM, Boon N, Bodelier PLE. 2016.**
478 Biotic interactions in microbial communities as modulators of biogeochemical processes: methanotrophy
479 as a model system. *Frontiers in Microbiology* **7**:1285 DOI 10.3389/fmicb.
- 480 **Huang LF, Song LX, Xia XJ, Mao WH, Shi K, Zhou YH, Yu JQ. 2013.** Plant-soil feedbacks and soil
481 sickness: from mechanisms to application in agriculture. *Journal of Chemical Ecology* **39(2)**:232-242
482 DOI org/10.1007/s10886-013-0244-9.
- 483 **Ikunaga Y, Sato I, Grond S, Numaziri N, Yoshida S, Yamaya H, Hiradate S, Hasegawa M, Toshima H,**
484 **Koitabashi M, Ito M, Karlovsky P, Tsushima S. 2011.** *Nocardioides* sp. strain WSN05-2, isolated from
485 a wheat field, degrades deoxynivalenol, producing the novel intermediate 3-epi-deoxynivalenol. *Applied*
486 *microbiology and biotechnology* **89(2)**:419-427 DOI org/10.1007/s00253-010-2857-z.
- 487 **Lee S, Ka JO, Song HG. 2012.** Growth promotion of *Xanthium italicum* by application of rhizobacterial
488 isolates of *Bacillus aryabhattai* in microcosm soil. *The Journal of Microbiology* **50(1)**:45-49 DOI
489 org/10.1007/s12275-012-1415-z.
- 490 **Li P, Dai C, Wang X, Zhang T, Chen Y. 2012.** Variation of soil enzyme activities and microbial community
491 structure in peanut monocropping system in subtropical China. *African Journal of Agricultural*
492 *Research* **7(12)**:1870-1879 DOI 10.5897/AJAR11.1713.
- 493 **Li XG, Ding CF, Hua K, Zhang TL, Zhang YN, Zhao L, Yang Y, Liu JG, Wang XX. 2014a.** Soil sickness
494 of peanuts is attributable to modifications in soil microbes induced by peanut root exudates rather than to
495 direct allelopathy. *Soil Biology and Biochemistry* **78**:149-159 DOI org/10.1016/j.soilbio.2014.07.019.
- 496 **Li XG, Ding CF, Liu JG, Zhang TL, Wang XX. 2015.** Evident response of the soil nematode community to
497 consecutive peanut monoculturing. *Agronomy Journal* **107(1)**:195-203 DOI 10.2134/agronj14.0257.
- 498 **Li XG, Ding CF, Zhang TL, Wang XX. 2014b.** Fungal pathogen accumulation at the expense of plant-
499 beneficial fungi as a consequence of consecutive peanut monoculturing. *Soil Biology and Biochemistry*

72:11-18 DOI org/10.1016/j.soilbio.2014.01.019.

Liu W, Wang Q, Wang B, Wang X, Franks AE, Teng Y, Li Z, Luo Y. 2015. Changes in the abundance and structure of bacterial communities under long-term fertilization treatments in a peanut monocropping system. *Plant and Soil* **395(1-2)**:415-427 DOI 10.1007/s11104-015-2569-3.

Liu X, Li Y, Han B, Zhang Q, Zhou K, Zhang X, Hashemi M. 2012. Yield response of continuous soybean to one-season crop disturbance in a previous continuous soybean field in Northeast China. *Field Crops Research* **138**:52-56 DOI 10.1016/j.fcr.2012.09.012.

Mellbye BL, Spieck E, Bottomley PJ, Sayavedra-Soto LA. 2017. Acyl-homoserine lactone production in nitrifying bacteria of the genera *Nitrosospira*, *Nitrobacter*, and *Nitrospira* identified via a survey of putative quorum-sensing genes. *Applied and Environmental Microbiology* **83(22)**: e01540-17 DOI 10.1128/AEM.01540-17.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* **41**:590–596 DOI 10.1093/nar/gks1219.

Ramesh A, Sharma SK, Sharma MP, Yadav N, Joshi OP. 2014. Inoculation of zinc solubilizing *Bacillus aryabhatai* strains for improved growth, mobilization and biofortification of zinc in soybean and wheat cultivated in Vertisols of central India. *Applied Soil Ecology* **73**:87-96 DOI org/10.1016/j.apsoil.2013.08.009.

Santoyo G, Orozco-Mosqueda MDC, Govindappa M. 2012. Mechanisms of biocontrol and plant growth-promoting activity in soil bacterial species of *Bacillus* and *Pseudomonas*: a review. *Biocontrol Science and Technology* **22(8)**:855-872 DOI org/10.1080/09583157.2012.694413.

Sharma SK, Ramesh A, Sharma MP, Joshi OP, Govaerts B, Steenwerth KL, Karlen DL. 2010. Microbial community structure and diversity as indicators for evaluating soil quality. *Biodiversity, Biofuels, Agroforestry and Conservation Agriculture* 317-358 DOI org/10.1007/978-90-481-9513-8_11.

- 524 **Takagi K, Fujii K, Yamazaki K, Harada N, Iwasaki A. 2012.** Biodegradation of melamine and its hydroxy
525 derivatives by a bacterial consortium containing a novel *Nocardioides* species. *Applied Microbiology and*
526 *Biotechnology* **94(6)**:1647-1656 DOI org/10.1007/s00253-011-3673-9.
- 527 **Uria AR, Piel J, Wakimoto T. 2018.** Biosynthetic Insights of Calyculin-and Misakinolide-Type Compounds
528 in “*Candidatus Entotheonella* sp.” *Methods in enzymology Academic Press* **604**: 287-330 DOI
529 org/10.1016/bs.mie.2018.02.017.
- 530 **van der Heijden MG, Wagg C. 2013.** Soil microbial diversity and agro-ecosystem functioning. *Plant and*
531 *Soil* **363(1-2)**:1-5 DOI org/10.1007/s11104-012-1321-5.
- 532 **Van der Putten WH, Bardgett RD, Bever JD, Bezemer TM, Casper BB, Fukami T, Kardol P,**
533 **Klironomos JN, Kulmatiski A, Schweitzer JA, Suding KN, Van de Voorde TFJ, Wardle DA. 2013.**
534 Plant–soil feedbacks: the past, the present and future challenges. *Journal of Ecology* **101(2)**:265-276 DOI
535 10.1111/1365-2745.12054.
- 536 **Vukicevich E, Lowery T, Bowen P, Úrbez-Torres JR, Hart M. 2016.** Cover crops to increase soil microbial
537 diversity and mitigate decline in perennial agriculture. A review. *Agronomy for Sustainable Development*
538 **36(3)**:48 DOI org/10.1007/s13593-016-0385-7.
- 539 **Wang HW, Sun K, Guan YX, Qiu MH, Zhang L, Dai CC. 2019.** Fungal endophyte *Phomopsis liquidambari*
540 biodegrades soil resveratrol: a potential allelochemical in peanut monocropping systems. *Journal of the*
541 *Science of Food and Agriculture* **99(13)**:5899-5909 DOI org/10.1002/jsfa.9865.
- 542 **Wang MZ, Chen XN. 2005.** Obstacle and countermeasure of sustainable high yield for peanut in low-hilly red
543 soil region. *Journal of Peanut Science* **34(2)**:17-22 DOI 10.14001/j.issn.1002-4093.2005.02.003.
- 544 **Wu L, Wang J, Huang W, Wu H, Chen J, Yang Y, Zhang Z, Lin W. 2015.** Plant-microbe rhizosphere
545 interactions mediated by *Rehmannia glutinosa* root exudates under consecutive monoculture. *Scientific*

Reports **5**:15871 DOI 10.1038/srep15871.

Xiong W, Zhao Q, Zhao J, Xun W, Li R, Zhang R, Wu H, Shen Q. 2015. Different continuous cropping spans significantly affect microbial community membership and structure in a vanilla-grown soil as revealed by deep pyrosequencing. *Microbial ecology* **70**(1):209-218 DOI 10.1007/s00248-014-0516-0.

Yang G, Ma K, Lu F, Wei CH, Dai XH. 2015. Effect of Continuous Cropping of Potato on Allelochemicals and Soil Microbial Community. *Journal of Ecology and Rural Environment* **5**:15 DOI 10.11934/j.issn.1673-4831. 2015.05. 015.

Zhou X, Liu J, Wu F. 2017. Soil microbial communities in cucumber monoculture and rotation systems and their feedback effects on cucumber seedling growth. *Plant and Soil* **415**:507–520 DOI 10.1007/s11104-017-3181-5.

Zhou X, Wang Z, Jia H, Li L, Wu F. 2018. Continuously monocropped *Jerusalem artichoke* changed soil bacterial community composition and ammonia-oxidizing and denitrifying bacteria abundances. *Frontiers in Microbiology* **9**:705 DOI org/10.3389/fmicb.2018.00705.

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

Figure legends

Figure 1. Overall abundance distribution of bacteria at the (A) class and (B) genus levels from soil that was monocropped with peanut.

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.

Figure 5. Heatmap presenting the distribution of the top 50 taxa at genus level in 16 monocropped peanut plots.

Figure 6. Relative abundance of significantly changed taxa at species level in plots that were monocropped with peanuts for different periods.

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.

Supporting Information

Figure S1.

File format: JPG

Title: Rarefaction curves for the 16S rRNA gene libraries constructed from each of the soil samples.

Figure S2.

File format: JPG

Title: Composition of soil bacteria at the phylum level: (A) abundance and (B) diversity compositions.

Figure S3.

File format: JPG

Title: Principal coordinate analysis (PCoA) of bacterial distributions in short- and long-term monocropped peanut soils for different peanut varieties.

Figure S4:

File format: JPG

Title: Heatmap showing the distribution of taxa at phylum level in 16 monocropped peanut plots.

Figure S5:

File format: JPG

Title: Relative abundance of significantly increased taxa at genus level in plots that were monocropped with peanuts for different periods.

Figure S6:

File format: JPG

Title: Relative abundance of significantly decreased taxa at genus level in plots that were monocropped with peanuts for different periods.

Figure S7:

File format: JPG

Title: Heatmap showing the distribution of the top 50 taxa at species level in 16 monocropped peanut plots.

Figure S8:

File format: JPG

Title: Composition of predicted bacteria functional pathways at KEGG level 1.

Figure S9:

File format: JPG

Title: Principal component analysis (PCA) of predicted bacterial functions in short- and long-term monocropped peanut soils for different peanut varieties: (A) KEGG level 3 and (B) KO level.

Figure 1

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

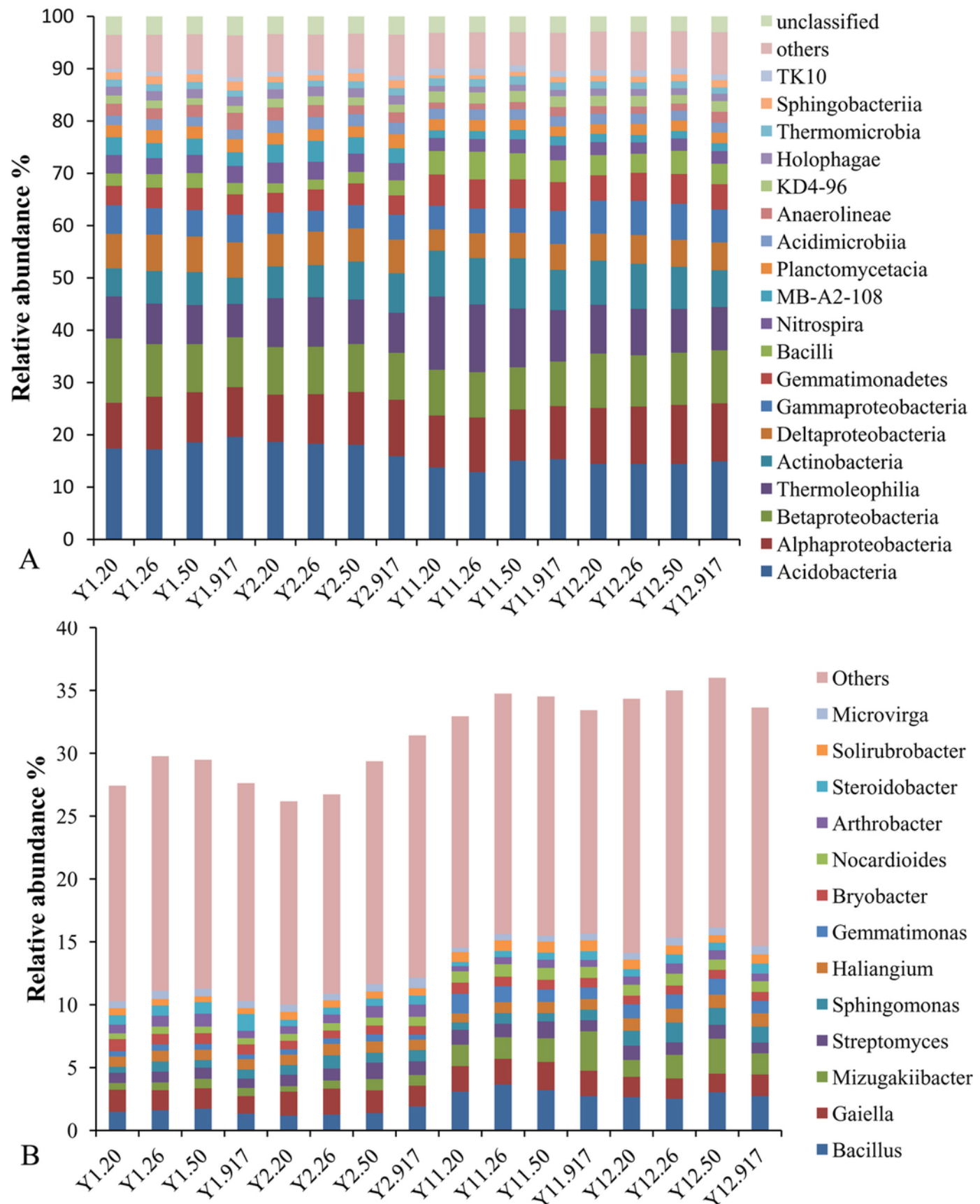


Figure 2

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

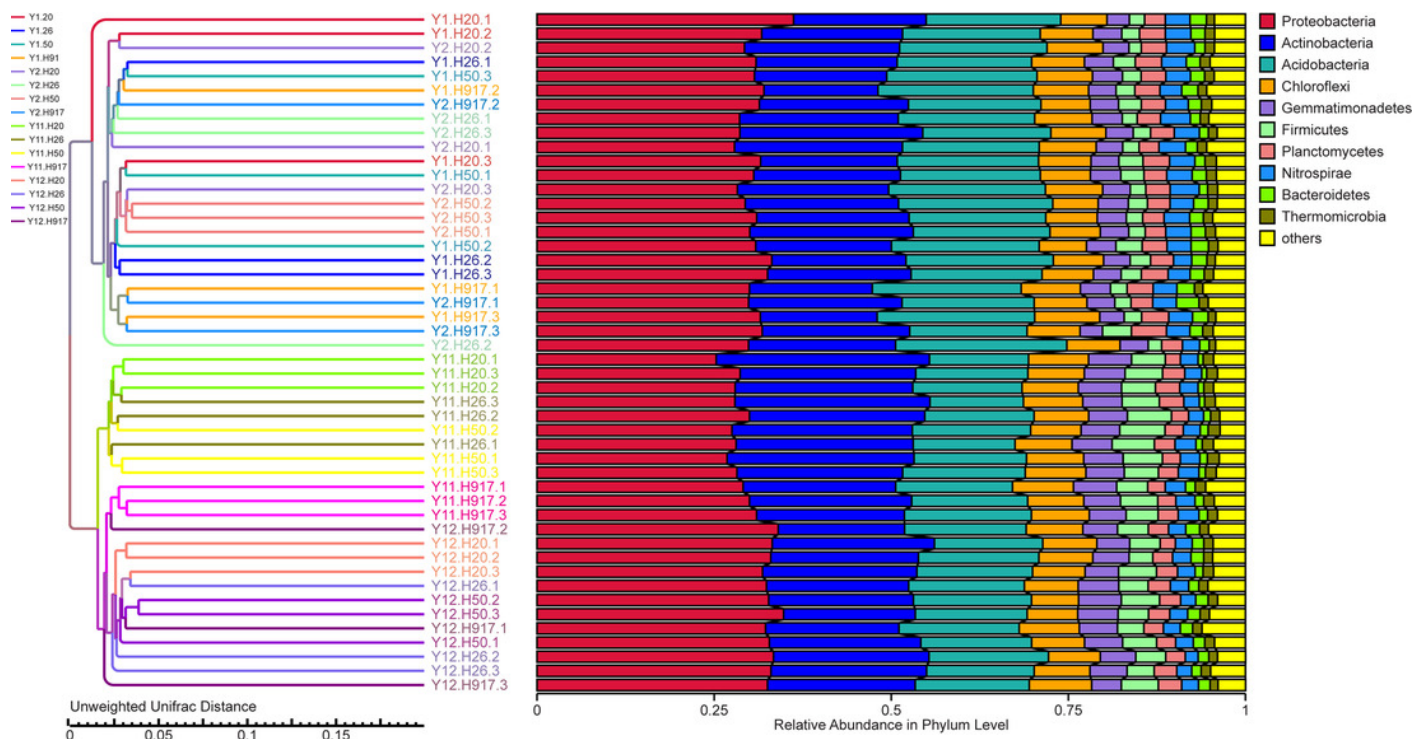


Figure 3

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

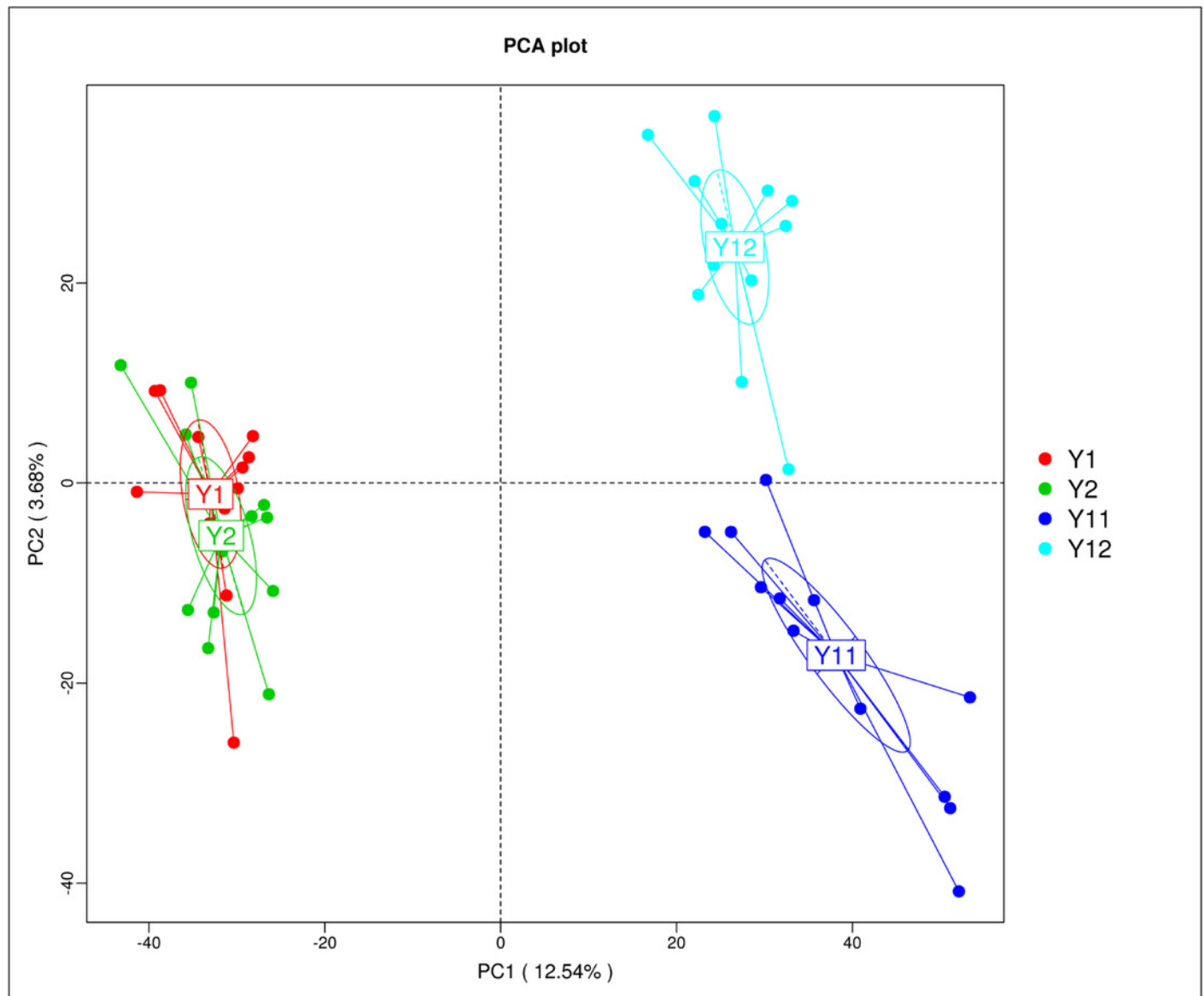


Figure 4

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

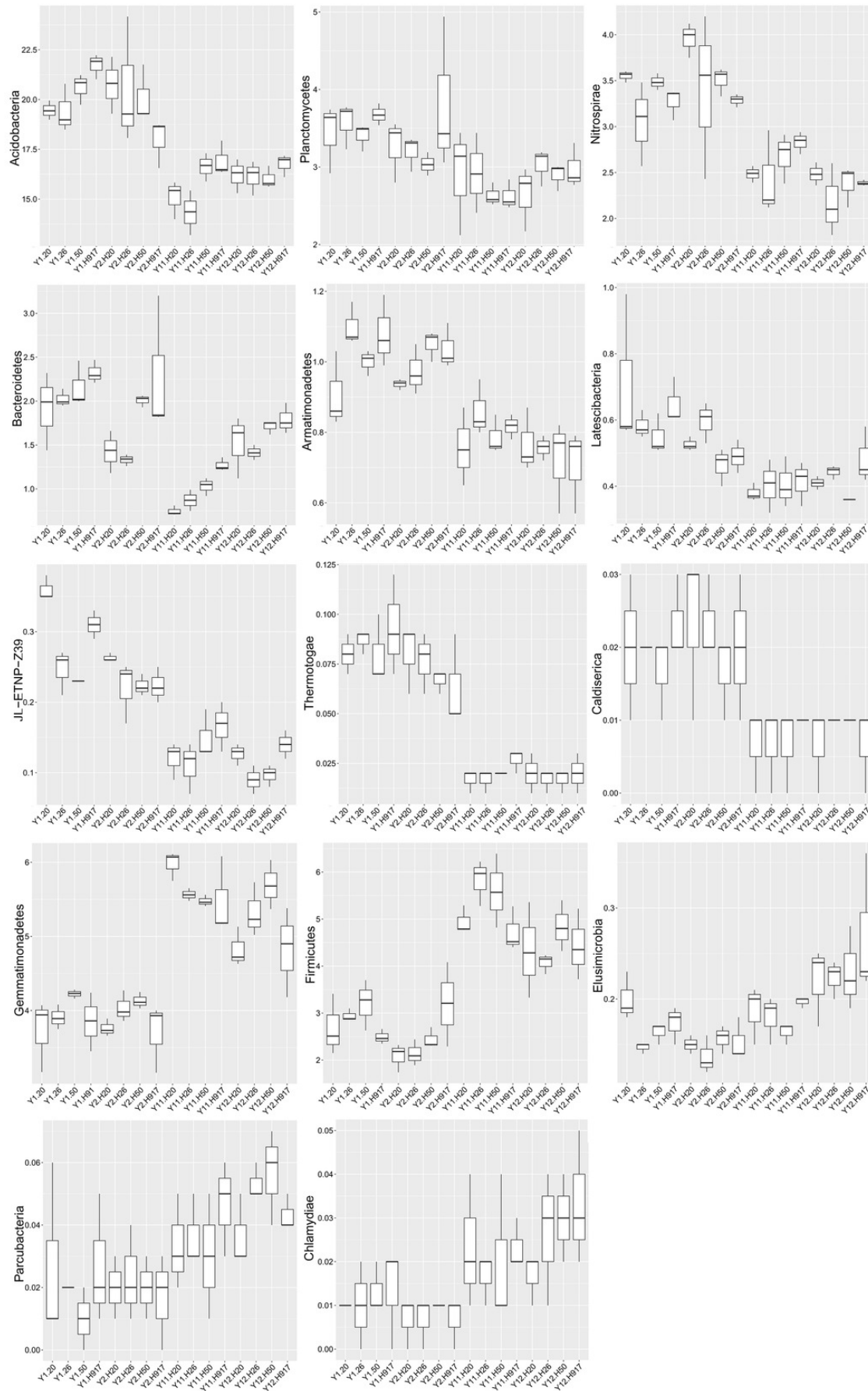


Figure 5

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

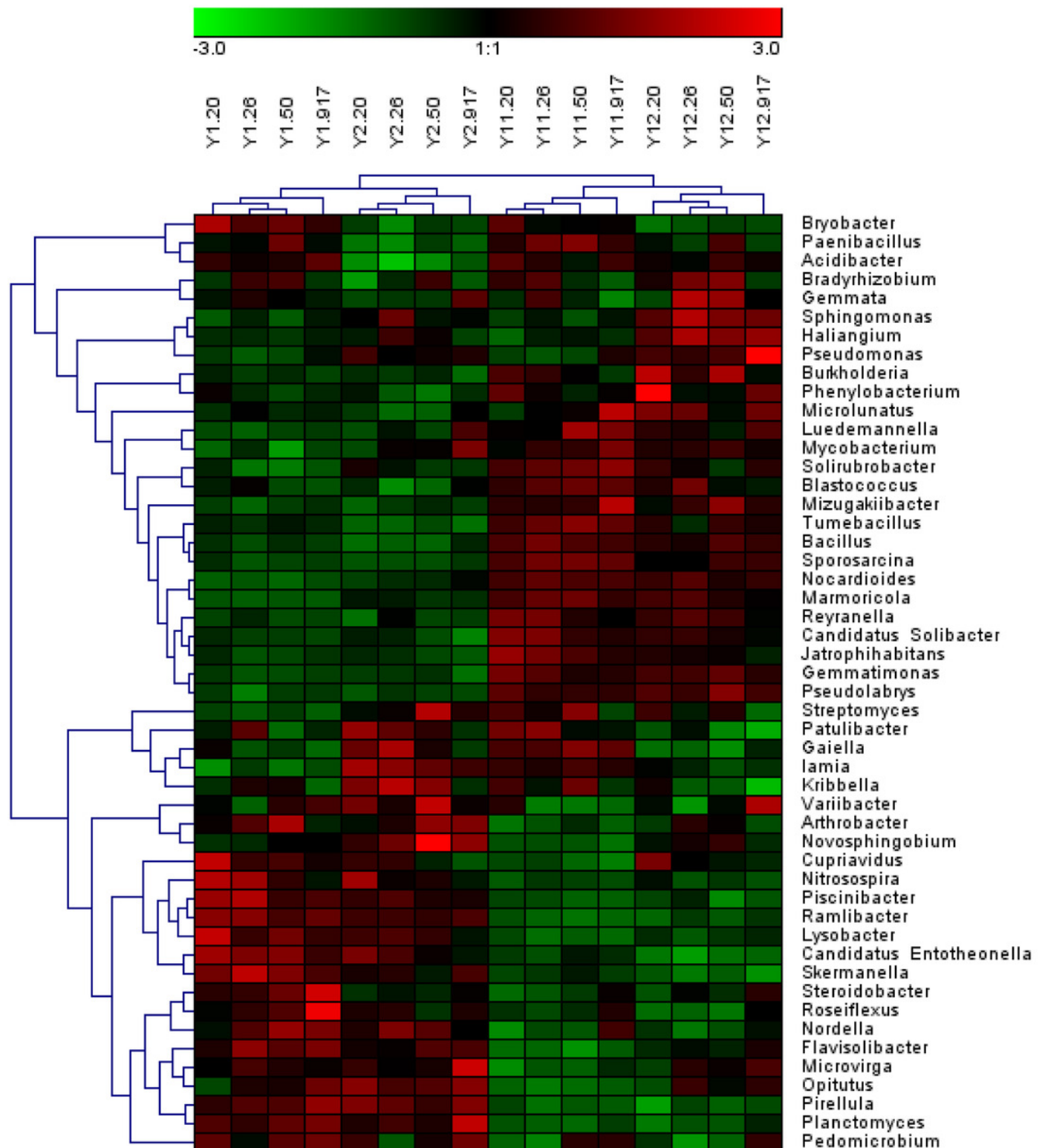


Figure 6

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

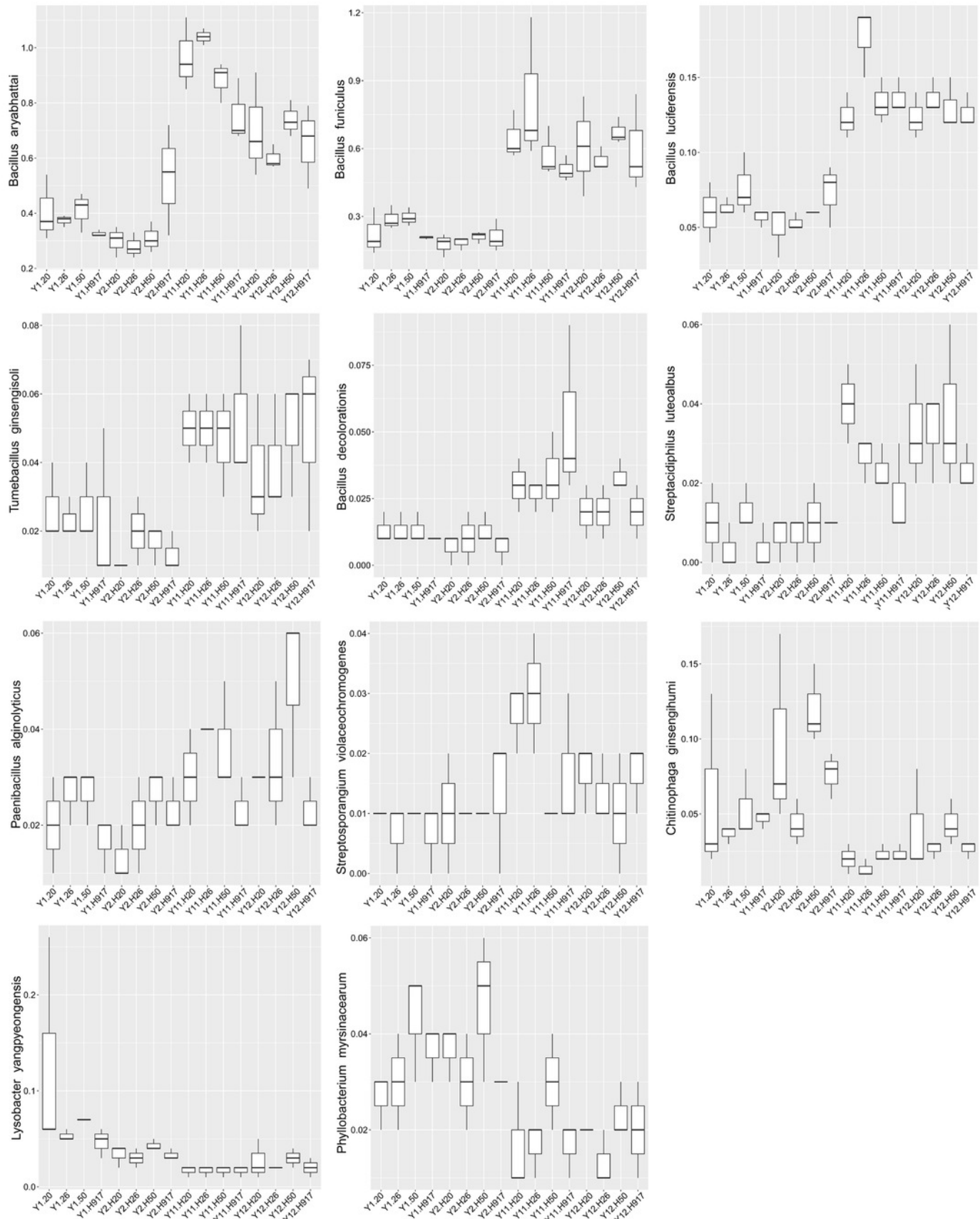


Figure 7

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

