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. A decrease in diversity and abundance of bacterial communities,

especially beneficial communities, and simplification of bacterial community function 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

45 growth or degrading potential soil allelochemicals, and should be closely related with soil
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49 the main cause of peanut soil sickness.

50 **INTRODUCTION**

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

60 Based on previous reports, there are four main factors contributing to soil sickness: disorder
61 in physicochemical soil properties, production, and accumulation of autotoxins, imbalance of soil
62 microbial communities and change in soil enzyme activity (*Huang et al., 2013; Zhou et al.,2018*).
63 Soil microorganisms that are critical to many soil biological, chemical, and physical processes
64 such as soil structure formation, mineral nutrition cycling, organic matter turnover and toxin
65 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.

Bacterial populations are most abundant and diverse in soil, and the interactions between soil, bacteria, and plants in root-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., 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 study aims 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 that had previously been planted with a wheat–maize rotation, thoroughly mixed, and then added to the pools. In order to construct the gradient of continuous monocropping plots, sweet potatoes were rotated with peanut in some plots during the experiment. 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. After harvest, the plots lay fallow until the next planting season. The distance between rows was 16.5 cm and the ridge width was 70 cm, with two peanut seeds per hole. Before planting, about 300 kg ha^{-1} of urea, 750 kg ha^{-1} of calcium magnesium phosphate, and 225 kg ha^{-1} of potassium chlorate were applied as fertilizer. The weeds were controlled using glyphosate (41% active ingredient in 3 L ha^{-1}) before planting. Plastic film mulching was applied during cultivation. 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.

According to our previous study (*Jiao et al., 2015*), 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. There were three replicates for each treatment. The bulk soils were collected at the full-bloom stage and five randomly selected replicate test plants were used for each sample. The soils around individual plants were sampled using a soil probe (1.5 cm diameter) at 5–10 cm soil depth, and at distances of 3–5, 8–10, and 13–15 cm from the main root. The root zone soils were then mixed together. These distances were chosen because the roots at this depth and these distances were relatively abundant and the soil microbial community around the peanut root system could be well characterized (Chen *et al*., 2012). Physico-chemical properties of pH, organic matter, and available nitrogen (N), phosphorus (P), and potassium (K) in the soil samples were determined using routine methods (Lu 1999). Samples Y1, Y2, Y11, and Y12 were for monocropping of 1, 2, 11, and 12 years, respectively; H20, H26, H50, and H917 were samples for four peanut varieties with distinct responses to monocropping.

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

Physico-chemical properties of soil

Physico-chemical properties of pH, organic matter, and available N, P, and K in the peanut monocropping soil were tested and analyzed (Table S1). The soil pHs in samples from 2-year monocropped plots (8.30–8.59) were slightly higher than those from 1-year monocropped plots (7.20–8.05), but were obviously lower in samples from 11- and 12-year monocropped plots

(6.46–6.92). The contents of available P were lower in long-term (15.12–21.12 mg kg^{-1}) than in short-term monocropping samples (39.37–48.58 mg kg^{-1}). The contents of available K were also lower in long-term (59.98–86.13 mg kg^{-1}) than in short-term monocropping samples (99.42–120.67 mg kg^{-1}). However, contents of organic matter and available N showed no obvious changes with long-term peanut monocropping.

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. 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.

212 **Bacterial community structure and composition**

213 In total, 99.73% of the identified 8856 OTUs were related to bacteria, and the other 24
214 OTUs accounting for 0.15% of the total quality-filtered sequences were affiliated with Archaea.
215 There were 41 bacteria phyla detected across all samples and 0.73% of bacterial sequences were
216 unclassified at the phylum level. The top 10 phyla accounted for 95.49% of the total sequences
217 and 79.67% of the total OTUs. The dominant phyla across all samples were Proteobacteria,
218 Actinobacteria, Acidobacteria, and Chloroflexi, accounting for 30.71, 21.59, 18.04, and 7.69% of
219 the total sequences, respectively, and 26.61, 7.43, 6.49, and 10.77% of the total 8856 OTUs,
220 respectively. The phyla Gemmatimonadetes, Firmicutes, Planctomycetes, Nitrospirae,
221 Bacteroidetes, and Thermomicrobia were relatively abundant and diverse, accounting for 4.66,
222 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,
223 3.97, and 1.19% of the total OTUs, respectively (Figure S2).

224 At the class level, 92 bacteria taxa were identified. The dominant 19 taxa (relative
225 abundance > 1%) accounted for >88.43% of the sequences in each sample. The most abundant
226 taxa (relative abundance > 5%) were Acidobacteria (16.19%), Alphaproteobacteria (10.11%),
227 Betaproteobacteria (9.48%), Thermoleophilia (9.22%), Actinobacteria (7.31%),
228 Deltaproteobacteria (5.77%), and Gammaproteobacteria (5.29%). The other relatively abundant
229 taxa included Gemmatimonadetes, Bacilli, Nitrospira, MB-A2-108, Planctomycetacia,
230 Acidimicrobiia, Anaerolineae, KD4-96, Holophagae, Thermomicrobia, Sphingobacteriia, and
231 TK10, each accounting for 1.05–4.66% of the total sequences (Figure 1A).

232 At the genus level, 494 taxa were detected, accounting for 31.41% of the total sequences;
233 and the top 50 taxa accounted for 75.04% of the total identified sequences at the genus level. The
234 dominant genera (relative abundance > 1%) were *Bacillus*, *Gaiella*, *Mizugakiibacter*, and
235 *Streptomyces*, accounting for 2.22, 1.79, 1.35, and 1.01% of the total sequences, respectively.
236 Relatively abundant genera were *Sphingomonas* (0.90%), *Haliangium* (0.89%), *Gemmatimonas*
237 (0.80%), *Bryobacter* (0.76%), *Nocardioides* (0.74%), *Arthrobacter* (0.71%), *Steroidobacter*
238 (0.68%), *Solirubrobacter* (0.66%), and *Microvirga* (0.57%) (Figure 1B).

239 Bacterial Community Variation at Different Levels with Monocropping Time

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

251 The heatmap analyses showed that soil bacterial community composition was significantly

252 diverse at different levels between short- and long-term monocropping. At the phylum level,
253 among the 41 bacteria taxa identified, nine taxa showed an obvious decrease in abundance with
254 monocropping time and five taxa showed an obvious increase in abundance (Figure S4, Figure 4).
255 The average abundance of Acidobacteria accounted for 20.30 and 19.83% in the 1- and 2-year
256 libraries, respectively, and decreased to 15.74 and 16.28% in the 11- and 12-year libraries. The
257 phyla Planctomycetes, Nitrospirae, and Bacteroidetes were relatively abundant in the 1- and 2-
258 year libraries, accounting for 3.12, 2.96, and 1.62% of the total sequences, respectively, and
259 correspondingly decreased in the 11- and 12-year libraries by 20.23, 17.47, and 27.81%.
260 Additionally, the taxa Armatimonadetes, Latescibacteria, JL-ETNP-Z39, Thermotogae, and
261 Caldiserica, for which relative abundances were <1%, also had clear decreasing tendencies. In
262 general, decreases in their abundance were accompanied by decreases in their diversity (Table
263 S2). In contrast, the abundance of some taxa increased with monocropping time. The average
264 abundance of Gemmatimonadetes, Firmicutes, and Elusimicrobia accounted for 3.91, 2.65, and
265 0.16% in the short-term monocropping libraries, respectively, and correspondingly increased to
266 5.40, 4.84, and 0.21% for the long-term. The other taxa with increasing abundance all had
267 relatively low abundance, accounting for 0.01–0.04% of the total sequences, and included
268 Parcubacteria and Chlamydiae. The diversity of the three relatively abundant taxa
269 (Gemmatimonadetes, Firmicutes, and Elusimicrobia) did not show obvious changes with
270 monocropping time, but that of the other low-abundance taxa tended to increase with
271 monocropping time (Table S2).

272 Analysis of the microbial community at lower taxonomic levels (genus or species) can

provide better phylogenetic resolution than at higher taxonomic levels (*Ho et al., 2016*). At the 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 obviously changed with

294 monocropping time (Figure S7, Figure 6). Among the top 10 most-abundant species, *Bacillus*
295 *aryabhattai* and *Bacillus funiculus* accounting for 0.58 and 0.42% of the total sequences,
296 respectively, showed increased abundance in long- compared to short-term monocropping
297 libraries. The increase rate of these two taxa reached 116.23 and 177.70%, respectively. The
298 relatively abundant taxa of *Bacillus luciferensis*, *Tumebacillus ginsengisoli*, *Bacillus*
299 *decolorationis*, and *Streptacidiphilus luteoalbus*, accounting for 0.01–0.10% of the total
300 sequences, increased their relative abundances by >118%. The taxa *Paenibacillus alginolyticus*
301 and *Streptosporangium violaceochromogenes* also presented increased abundance with increase
302 rates ranging within 52.07–84.49%, but the change trends differed among peanut varieties. The
303 species *Paenibacillus alginolyticus* and *Streptosporangium violaceochromogenes* showed no
304 obvious changes in abundance in Huayu 917 and Huayu 50 libraries, respectively. In contrast to
305 the above taxa, *Chitinophaga ginsengihumi*, *Lysobacter yangpyeongensis*, and *Phyllobacterium*
306 *myrsinacearum*, accounting for 0.03–0.05% of the total sequences, showed obvious decreases in
307 abundance and the abundance decrease rate ranged within 32.57–61.59%.

308 **Prediction of bacteria community functions**

309 The predominant category of the predicted functional genes was affiliated with metabolism
310 (48.71%), followed by genetic information (21.03%), environmental information processing
311 (12.89%), and cellular processes (7.62%) (Figure S8). The PCA was used to cluster the predicted
312 functional pathway within soil samples at three KEGG levels. The analyses at KEGG levels 2
313 and 3 and KEGG ortholog (KO) level all showed similar clusters to that of bacterial community

314 structure (Figure 7, Figure S9). Samples from 1- and 2-year monocropping plots were grouped
315 into one class, and samples from 11- and 12-year plots were grouped into another.

316 Bacterial community functions also presented significant changes at different pathway
317 levels across long-term monocropping. For KEGG pathways at KEGG level 2 that are involved
318 in carbohydrate metabolism, endocrine system, excretory system, nucleotide metabolism,
319 transport and catabolism, transcription, biosynthesis of other secondary metabolites, and aging
320 showed decreasing trends with long-term monocropping, but pathways affiliated with membrane
321 transport and cellular community prokaryotes showed increased abundance (Table S3). There
322 were 390 KEGG pathways at KEGG level 3 predicted in our libraries. The abundant pathways
323 (>1.0%) that presented obvious changes in abundance with monocropping period were further
324 examined. The pathways involved in DNA repair and recombination proteins, purine metabolism,
325 transfer RNA biogenesis, exosome, amino acid related enzymes, pyrimidine metabolism,
326 mitochondrial biogenesis, ribosome, oxidative phosphorylation, carbon fixation pathways in
327 prokaryotes, aminoacyl tRNA biosynthesis as well as alanine, aspartate, and glutamate
328 metabolism showed decreased abundance in long- compared to short-term monocropping
329 samples. In contrast, pathways involved in ribosome biogenesis, messenger RNA biogenesis,
330 RNA degradation, quorum sensing, glyoxylate and dicarboxylate metabolism, and lipid
331 biosynthesis proteins were enriched in long-term monocropping samples (Table S4).

332 **DISCUSSION**

333 It has been demonstrated that soil microorganisms play influential roles in the productivity
334 and sustainability of agricultural systems (*Van der Heijden and Wagg, 2013; Vukicevich et al.*

335 *2016*). 'Soil sickness' caused by continuous peanut monocropping could be closely related to the
336 dynamics of soil microbial communities. Our previous study using library analysis showed that
337 soil microbial community structure shifted during three years of continuous peanut
338 monocropping (*Chen et al., 2012; Chen et al., 2014*). Li *et al*. reported that consecutive peanut
339 monoculture changed communities of soil nematodes and fungi (*Li et al., 2014b; Li et al., 2015*).
340 However, the specific dynamic succession of the bacterial community, which is most diverse in
341 soil, under long-term peanut monocropping is not clear.

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

349 Our previous study showed that bacterial community structure presented significant
350 dynamics during three years of peanut monocropping, but was less affected by peanut growth
351 stage (*Chen et al. 2014*). *Li et al*. (*2014b*) reported that fungal communities were significantly
352 selected by the monocropping period of peanut, but also were less affected by growth stage in
353 the red soil region of southern China. In the present study, the UPGMA, PCA, and PCoA
354 methods were used to cluster the bacterial communities within the 48 samples from short- and

355 long-term monocropping soil. The analyses showed that all samples regardless of variety from 1-
356 and 2-year monocropping plots were grouped into one class, and samples from 11- and 12-year
357 plots were grouped into another. However, the cluster distance between samples from 1- and 2-
358 year plots was shorter than that between samples from 11- and 12-year plots. Additionally, soil
359 pH and contents of available P and K in soil also obviously changed with long-term
360 monocropping of peanut. Monocropping time had a strong influence on the microbial
361 communities as well as physico-chemical properties, but peanut variety and growth stage had
362 little impact. Additionally, the soil bacterial community had significantly greater dynamics under
363 long- compared to short-term monocropping time.

364 Our previous study demonstrated that bacterial communities at different taxonomic levels
365 showed obvious dynamics during three years of peanut monocropping (*Chen et al. 2014*). Most
366 of the obviously changed taxa at the order level showed abundance and diversity declines with
367 monocropping time and only several taxa showed increased abundance and diversity (*Chen et al.*
368 *2014*). A third cropping of Jerusalem artichoke also decreased bacterial alpha-diversity compared
369 to 1–2 years of monocropping (*Zhou et al., 2018*). In this study, under long-term peanut
370 monocropping, the alpha-diversity of the soil bacterial community generally decreased. At the
371 phyla level, nine taxa that accounted for 0.01–18.04% of the total sequences showed obvious
372 decreases 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

obvious change in diversity 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 the genera level, *Steroidobacter*, *Candidatus Entotheonella*, *Nitrosospira*, *Pirellula*, *Piscinibacter*, *Ramlibacter*, *Lysobacter*, *Skermanella*, and *Planctomyces* showed decreased abundance with monocropping time. At the species level, *Chitinophaga ginsengihumi*, *Lysobacter yangpyeongensis*, and *Phyllobacterium myrsinacearum* also showed obvious decreases with long-term monocropping. Several taxa found were beneficial to plant growth. The genus *Nitrosospira* is reportedly related to nitrogen cycle progress (*Mellbye et al., 2017*) and *Candidatus Entotheonella* is related to biosynthesis (*Uria et al., 2018*). Additionally, at the species level, *Phyllobacterium*

396 *myrsinacearum* functions in nitrogen-fixing (*Gonzalez-Bashan et al., 2000*). Studies concerning
397 roles in plants of the other decreased taxa at the genus or species levels were not found. However,
398 this also demonstrated a decrease in abundance of the beneficial bacteria community with long-
399 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
435 long-term plots were grouped into another. Comparing analyses of the abundance indicated that
436 many detected KEGG pathways at KEGG levels 2 or 3 had obvious changes with long-term
437 monocropping. Most had a decreasing trend with long-term peanut monocropping, and only a

few showed an increase in abundance. 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 and contents of available P and K in soil also obviously changed with 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. A decrease in diversity and abundance of bacterial communities, especially beneficial communities, and simplification of bacterial community function 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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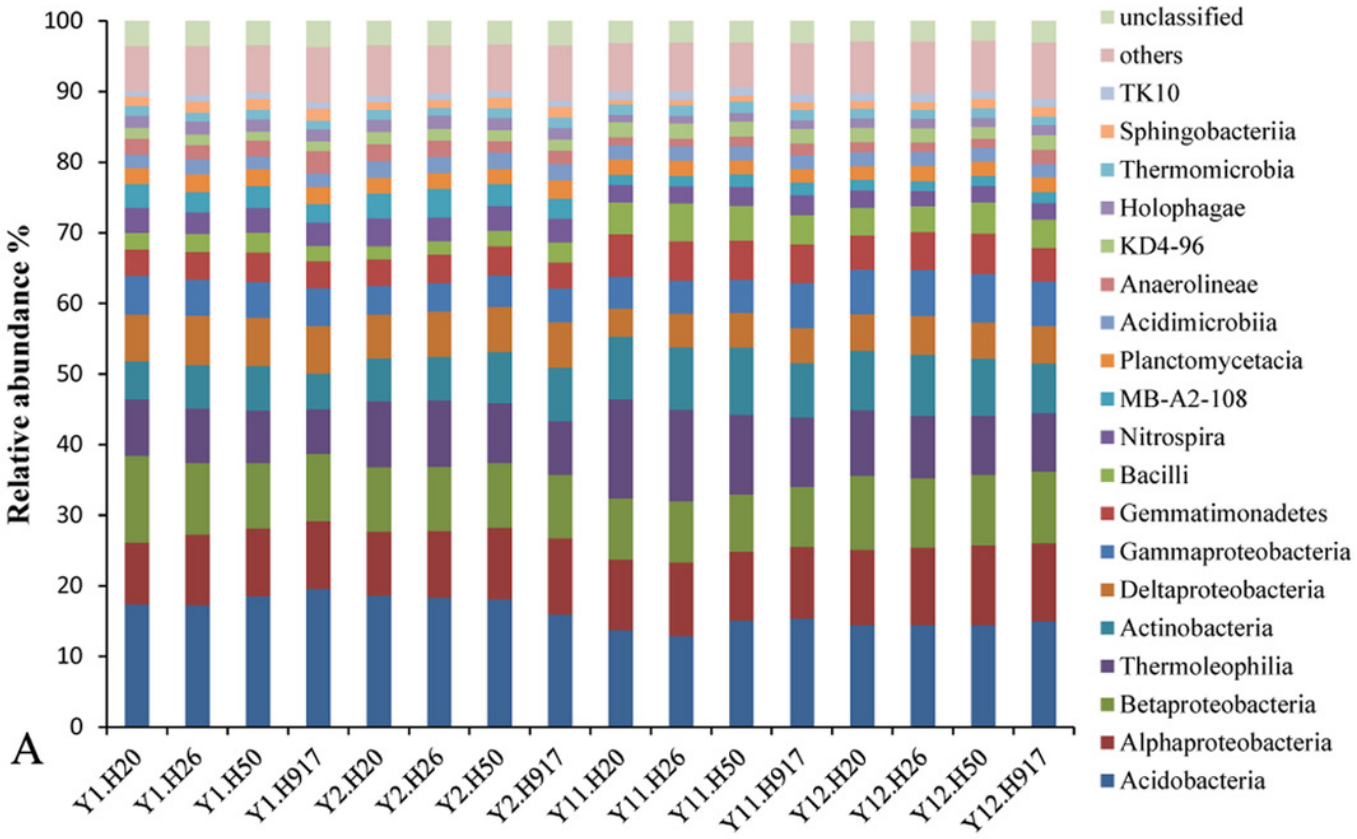
Figure 1

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


Relative abundance %
100
90
80
70
60
50
40
30
20
10
0
A
Y1.H20
Y1.H26
Y1.H50
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
unclassified
others
TK10
Sphingobacteriia
Thermomicrobia
Holophagae
KD4-96
Anaerolineae
Acidimicrobiia
Planctomycetacia
MB-A2-108
Nitrospira
Bacilli
Gemmatimonadetes
Gammaproteobacteria
Deltaproteobacteria
Actinobacteria
Thermoleophilia
Betaproteobacteria
Alphaproteobacteria
Acidobacteria

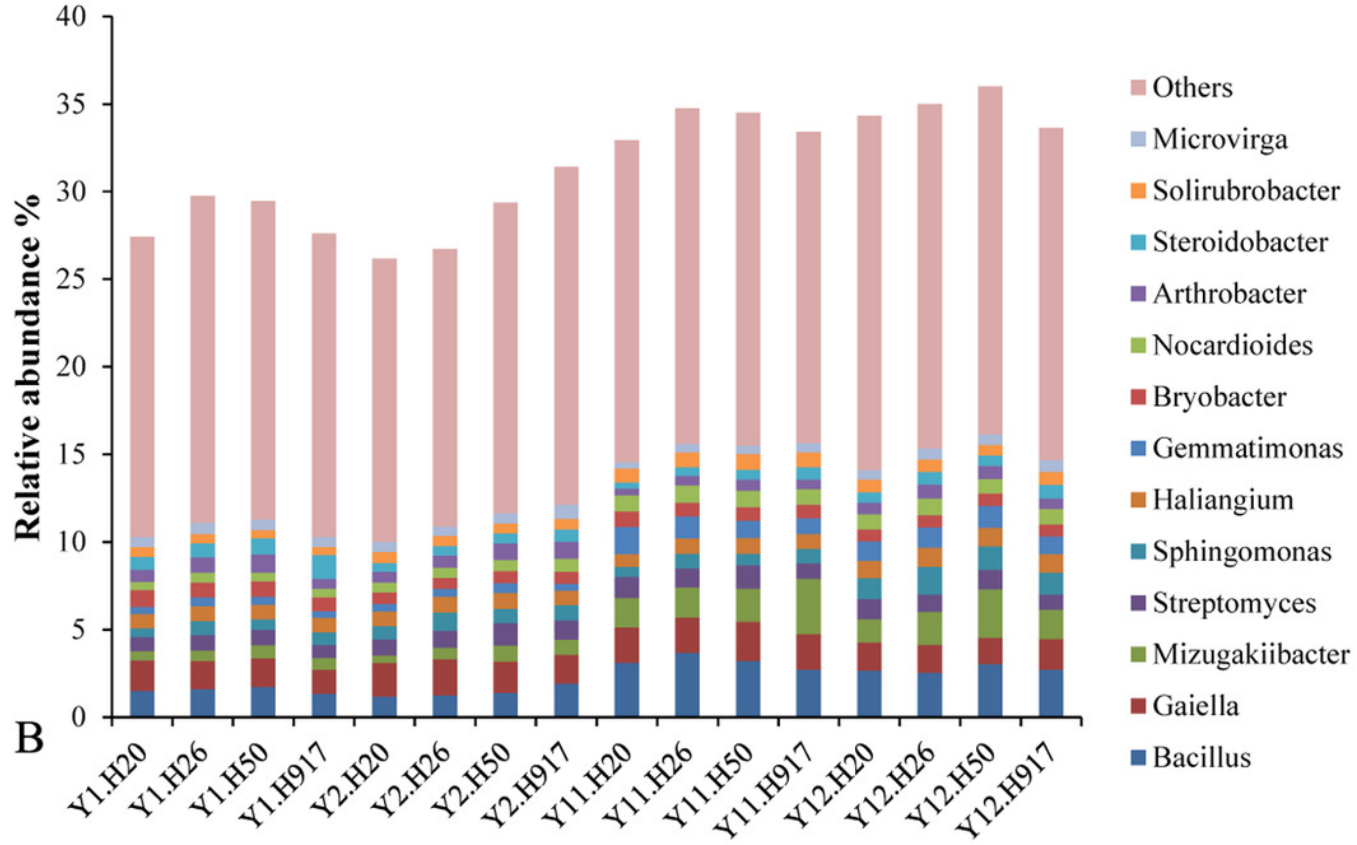

Relative abundance %
40
35
30
25
20
15
10
5
0
B
Y1.H20
Y1.H26
Y1.H50
Y1.H917
Y2.H20
Y2.H26
Y2.H50
Y2.H917
Y11.H20
Y11.H26
Y11.H50
Y11.H917
Y12.H20
Y12.H26
Y12.H50
Y12.H917
Others
Microvirga
Solirubrobacter
Steroidobacter
Arthrobacter
Nocardioides
Bryobacter
Gemmatimonas
Haliangium
Sphingomonas
Streptomyces
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.

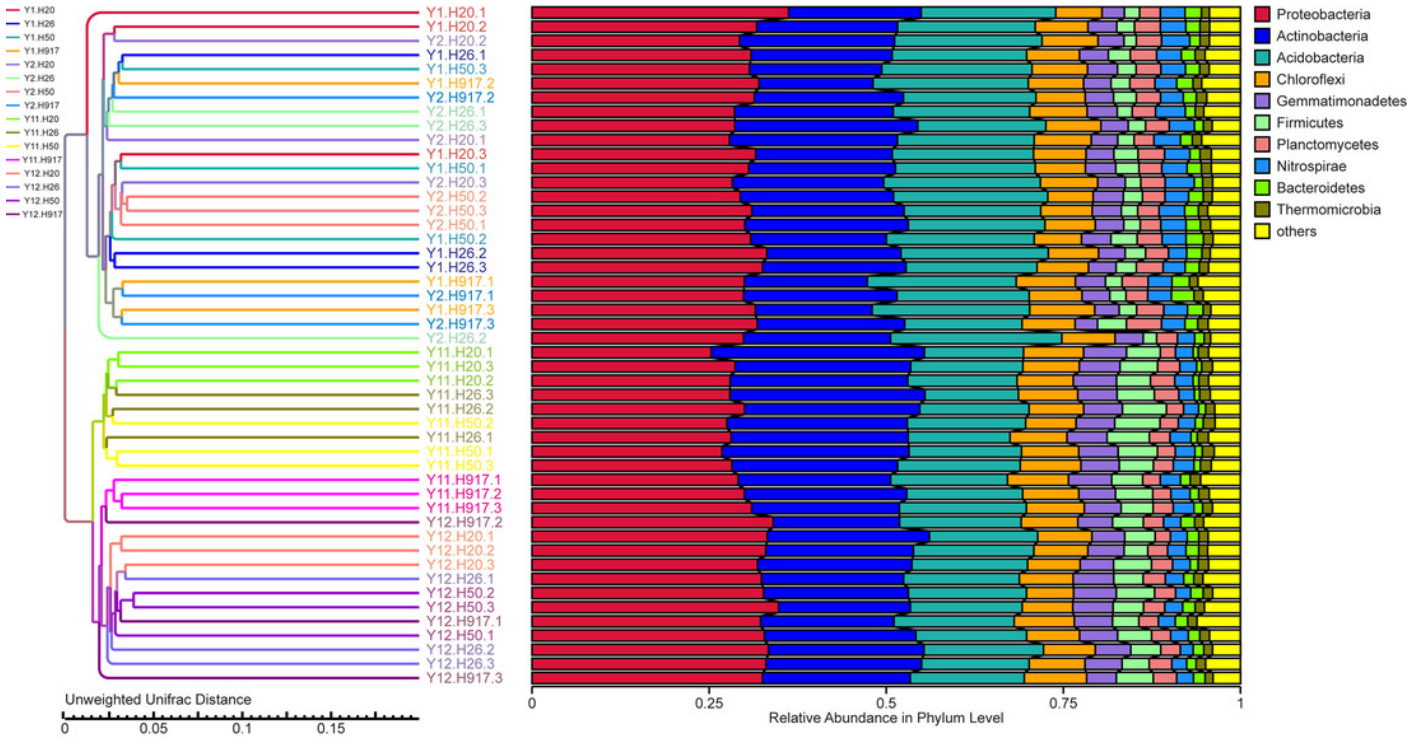


Figure 3

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

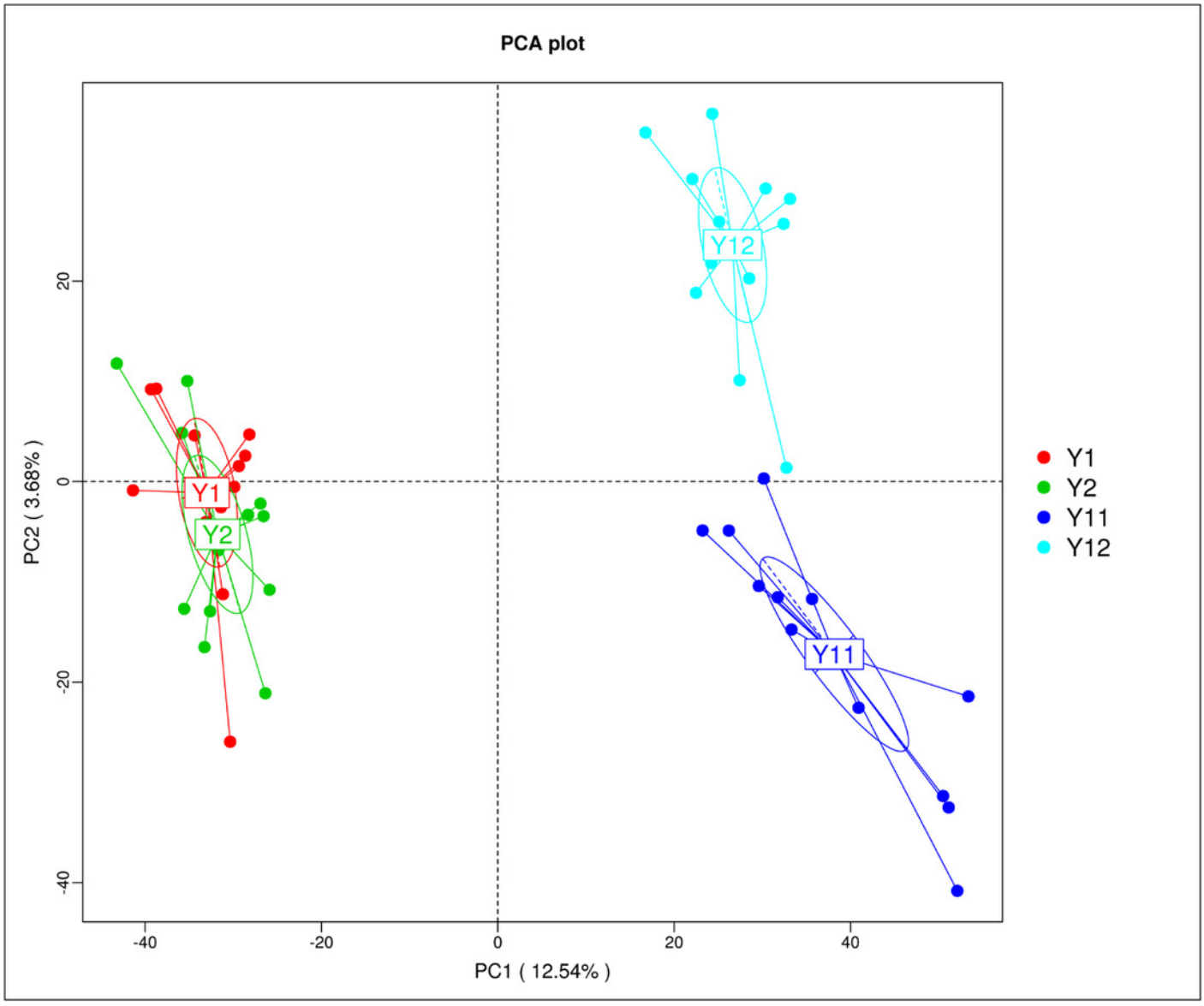


Figure 4

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

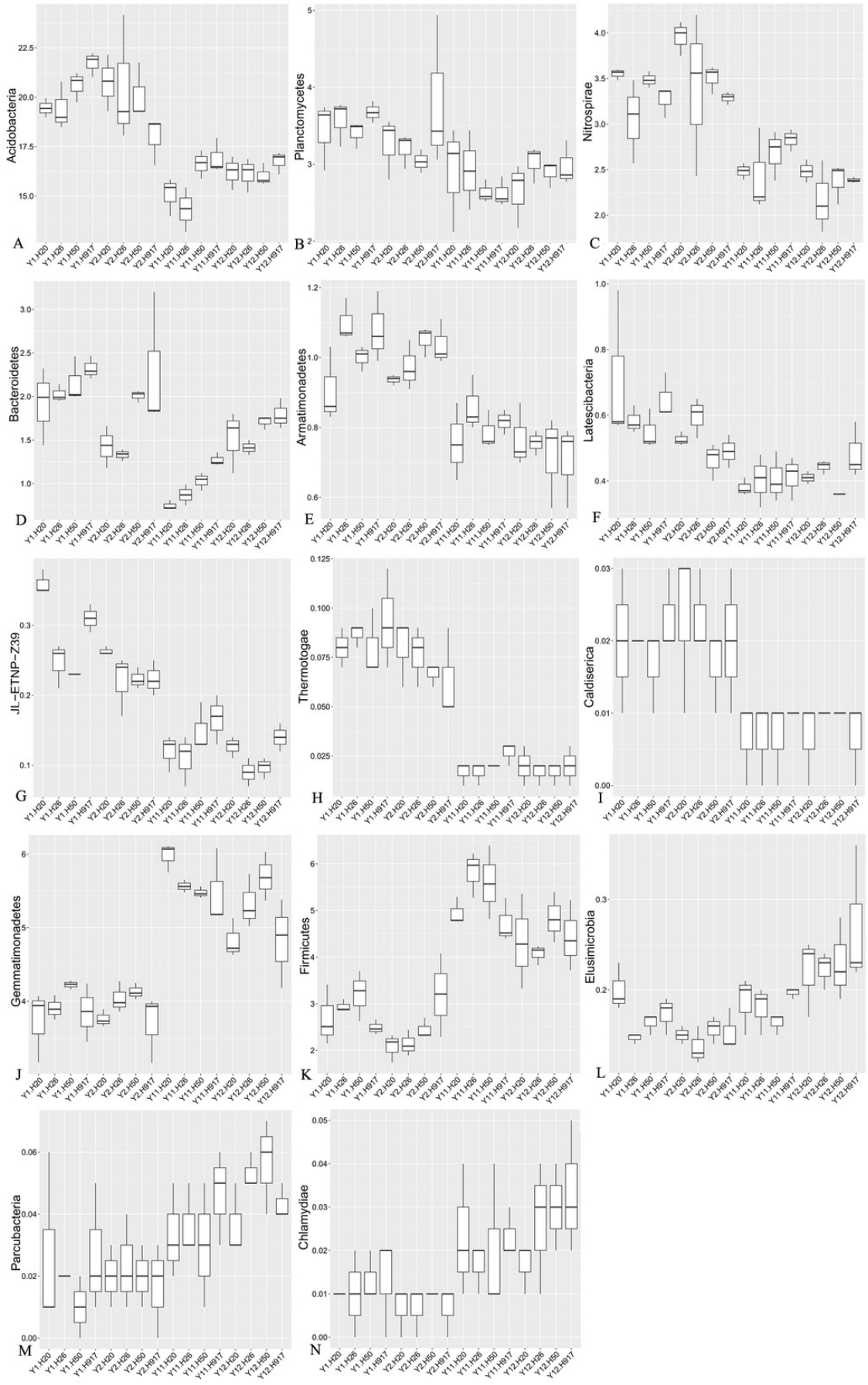

A
Acidobacteria
B
Planctomycetes
C
Nitrospirae
D
Bacteroidetes
E
Armatimonadetes
F
Latescibacteria
G
JL-ETNP-Z39
H
Thermotogae
I
Caldiserica
J
Gemmatimonadetes
K
Firmicutes
L
Elusimicrobia
M
Parcubacteria
N
Chlamydiae
Y1.H20
Y1.H26
Y1.H50
Y1.H917
Y2.H20
Y2.H26
Y2.H50
Y2.H917
Y11.H20
Y11.H26
Y11.H50
Y11.H917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

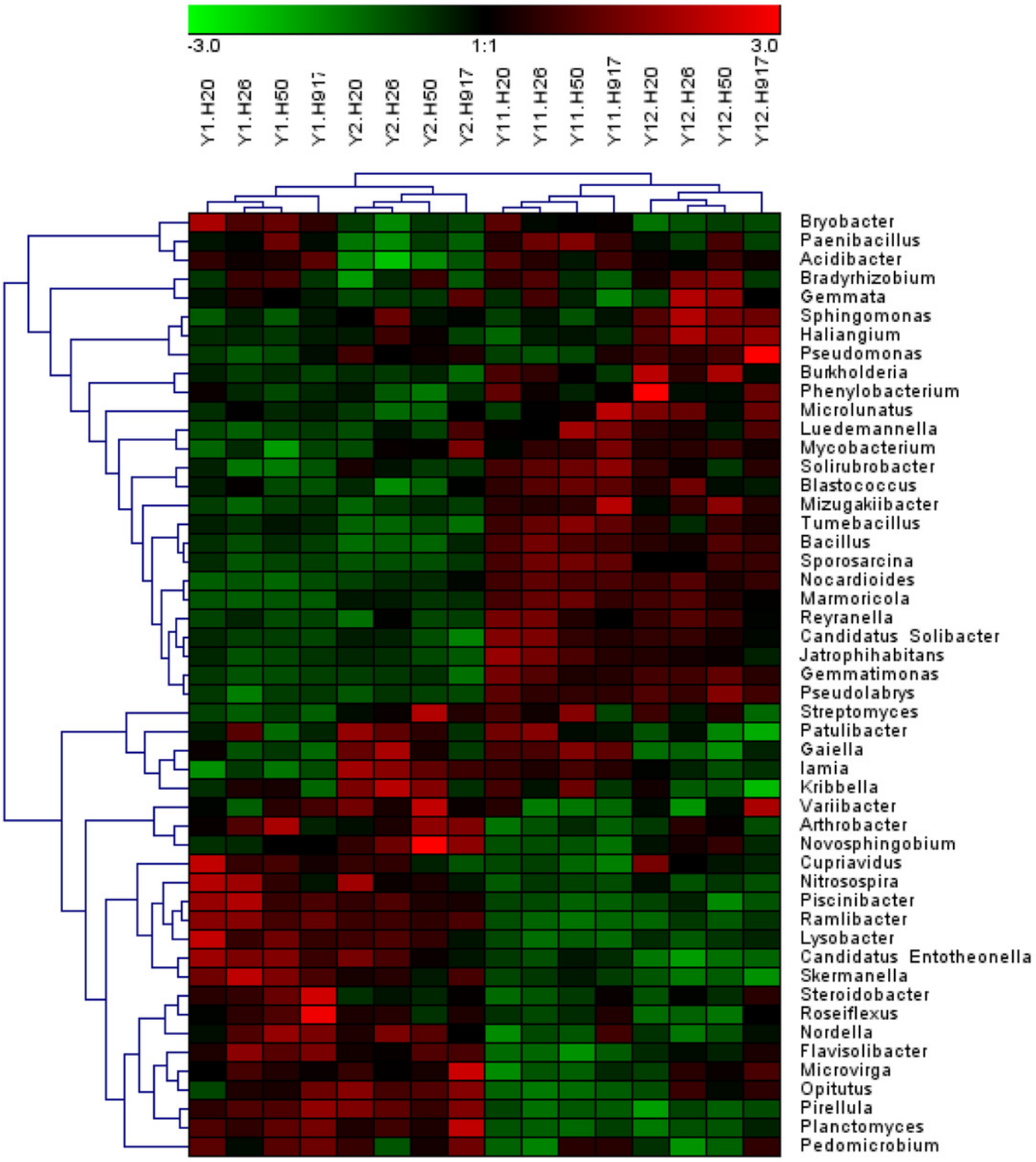

-3.0
1:1
3.0
Y1.H20
Y1.H26
Y1.H50
Y1.H917
Y2.H20
Y2.H26
Y2.H50
Y2.H917
Y11.H20
Y11.H26
Y11.H50
Y11.H917
Y12.H20
Y12.H26
Y12.H50
Y12.H917
Bryobacter
Paenibacillus
Acidibacter
Bradyrhizobium
Gemmata
Sphingomonas
Haliangium
Pseudomonas
Burkholderia
Phenylobacterium
Microlunatus
Luedemannella
Mycobacterium
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.

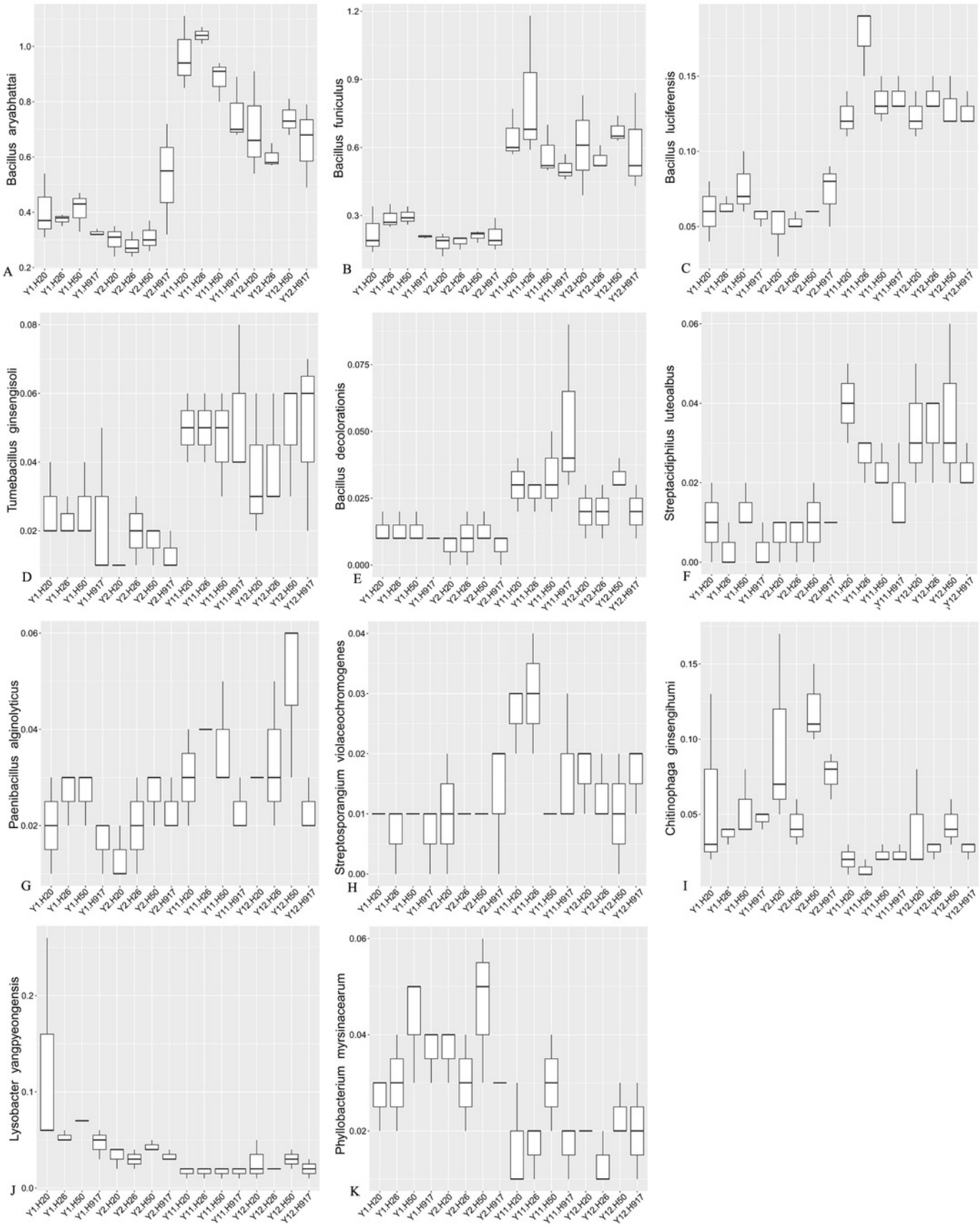

A
Bacillus aryabhattai
B
Bacillus funiculus
C
Bacillus luciferensis
D
Tumebacillus ginsengisoli
E
Bacillus decolorationis
F
Streptacidiphilus luteoalbus
G
Paenibacillus alginolyticus
H
Streptosporangium violaceochromogenes
I
Chitinophaga ginsengihumi
J
Lysobacter yangpyeongensis
K
Phyllobacterium myrsinacearum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

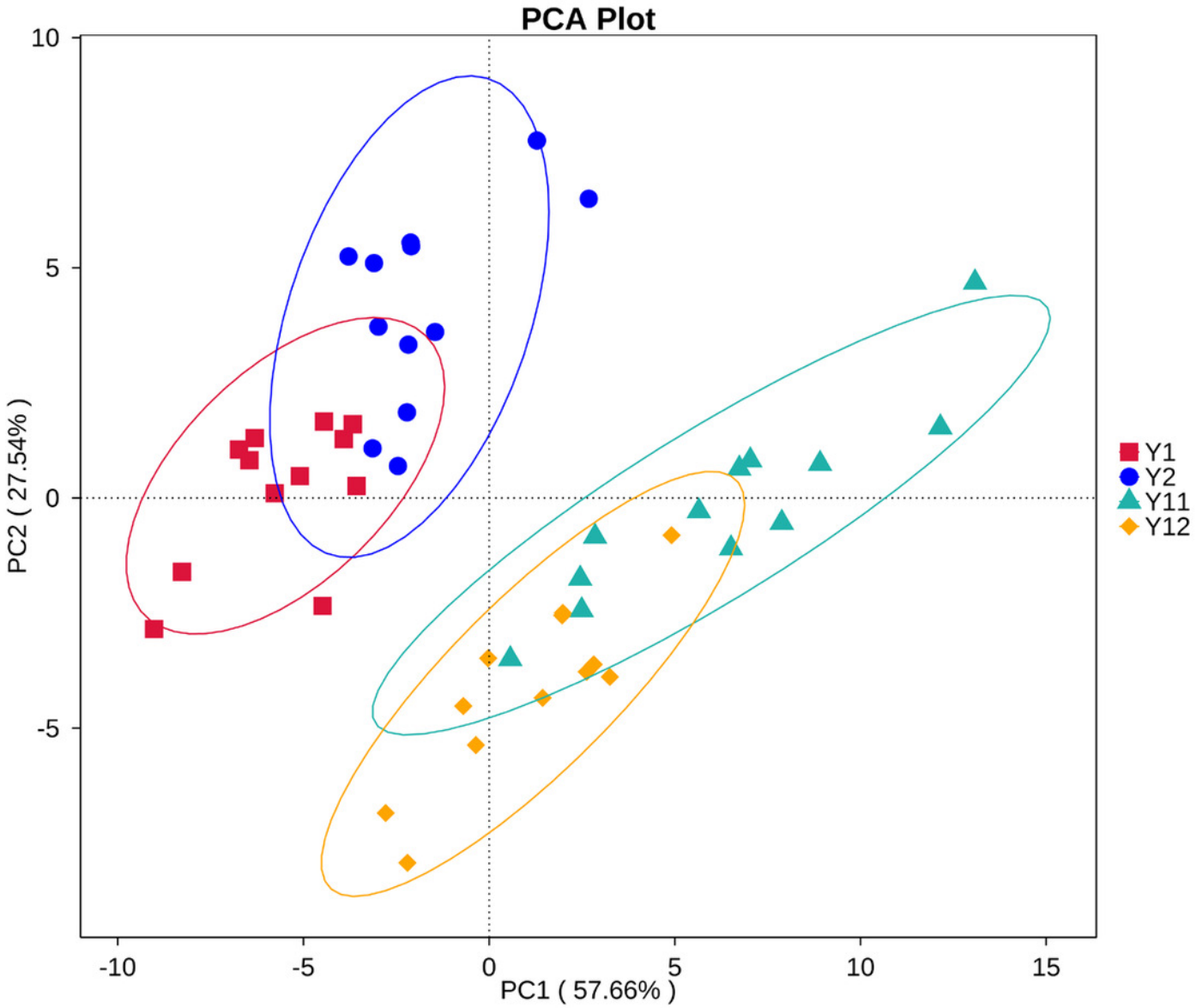


Table 1(on next page)

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