Continuous monocropping highly affect the composition and diversity of microbial communities in peanut (*Arachis hypogaea* L.)

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**Abstract**

Continuous cropping systems are the leading cause of decreased soil biological environments in terms of unstable microbial population and diversity index. Nonetheless, their responses to consecutive peanut monocropping cycles have not been thoroughly investigated. In this study, the structure and abundance of microbial communities were characterized using pyrosequencing-based approach in peanut monocropping cycles for three consecutive years. The results showed that continuous peanut cultivation led to a substantial decrease in soil microbial abundance and diversity from initial cropping cycle (T1) to later cropping cycle (T3). Peanut rhizosphere soil had Actinobacteria, Proteobacteria, and Gemmatimonadetes as the major bacterial phyla. Ascomycota, Basidiomycota were the major fungal phylum, while Crenarchaeota and Euryarchaeota were the most dominant phyla of archaea. Several bacterial, fungal and archael taxa were significantly changed in abundance under continuous peanut cultivation. Bacterial orders, Actinomycetales, Rhodospirillales and Sphingomonadales showed decreasing trends from T1>T2>T3. While, pathogenic fungi Phoma was increased and beneficial fungal taxa Glomeraceae decreased under continuous monocropping. Moreover, Archaeal order Nitrososphaerales observed less abundant in first two cycles (T1&T2), however, it increased in third cycle (T3), whereas, Thermoplasmata exhibit decreased trends throughout consecutive monocropping. Taken together, we have shown the taxonomic profiles of peanut rhizosphere communities that were affected by continuous peanut monocropping. The results obtained from this study pave ways towards a better understanding of the peanut rhizosphere soil microbial communities in response to continuous cropping cycles, which could be used as bioindicator to monitor soil quality, plant health and land management practices.

**Keywords:** Archaea population structure; continuous monocropping; microbial responses; peanut (*Arachis hypogaea* L.); pathogen fungi; rhizosphere bacterial community

**Introduction**

The rhizosphere microbiome is known to play an important role in fostering plant development, partially by combating soil-borne phyto parasites and improving nutrient uptake. These microorganisms have...
fundamental role in soil-based ecosystem functions and key processes including, organic matter turnover, mineral nutrition cycling, soil toxin removal or accumulation and manifest their effects by affecting soil health and plant nutrition (Nannipieri et al., 2003; Wardle et al., 2004; Ball, 2005). Soil microbial communities are affected by a wide range of factors, such as continuous monocropping (Zhao et al., 2020), soil management practices (Fournier et al., 2020; Rincon-Florez et al., 2020) and soil type (Xu et al., 2020). Consecutive monocropping, which means cultivation of the same crop in the same soil for several years, greatly affect microbial communities. The problems associated with continuous cropping are thought to be caused by three factors: nutrient imbalances in the soil, autotoxicity of root exudates, and shifts in the microbial community composition (Coskun et al., 2017). Majority of root exudates, comprising primary metabolites (sugars, amino acids, and organic acids), are thought to be passively released from the root tip and utilised by rhizosphere-dwelling microorganisms (Canarini et al., 2019). In rhizosphere, there is positive plant-soil feedback where plants benefit from root exudation by allowing self-roots and neighbour-roots to recognize one other, promoting nutrient uptake, root-symbiont relations and stimulating helpful microorganisms (e.g., symbionts) (Mommer et al., 2016; Meier et al., 2017). The build-up of phytotoxic chemicals in soil, on the other hand, causes a negative plant–soil feedback. This feedback limits beneficial microbiota while promoting parasite and disease outbreaks, resulting in autotoxicity or soil sickness, as well as reduction in plant growth and development (Harrison and Bardgett, 2010). Autotoxicity and soil sickness are common outcomes of negative plant–soil interactions, which are mostly caused by continuous monocropping. It is well recognized that root exudates and microbial diversity have a close association (Eisenhauer et al., 2017; Arafat et al., 2019), however, it is still unclear to what extent they impact one another. Microbial diversity is reported to be affected by exuded primary metabolites (Steinauer et al., 2016). During long-term continuous cropping, communities of beneficial soil microorganisms often undergo decline while communities that considered pathogenic increased (Arafat et al., 2019). Consequently, consecutive monoculture led to declines in yield and quality and ultimately increasing soil sickness or replanting disease (Larkin, 2003; Li et al., 2012; Huang et al., 2013). The issues related with consecutive monocropping, such as autotoxicity of root exudates, altered microbial diversity and shift in the community composition have been reported in both perennial and annual crops such as coffee (Zhao et al., 2018), tea (Arafat et al., 2017; Arafat et al., 2019), sugar beet (Huang et al., 2019), potato (Li et al., 2019a) and soybean (Tian et al., 2020). Previous studies have shown that soil fungal pathogenic communities increased with consecutive plant monoculture, resulting in increased disease pressure (Li et al., 2010; Chen et al., 2012; Arafat et al., 2019). It is believed that diversity and abundance of microbes dwelling in the soil is critical for sustainable soil health. Because of quick response to the environmental constrains, soil microbial communities can serve as the highly sensitive bioindicators of soil health, as they have close relationship with the soil condition and land management practices (Sharma et al., 2010). Consequently, assessing continuous monocropping effects on the microbial population is essential strategy to monitor soil quality, and land management. Also, management of rhizosphere microbes and preserving the equilibrium between beneficial and harmful microbes in the soil, particularly under monocropping systems, are critical to the plant growth and development.

Rhizosphere is the soil portion affected by the plant roots, is one of the most complex ecosystems on earth (Hartmann et al., 2008). This microecosystem is the “hot spot” for chemical contact, exchange of compound and nutrients between host and below-ground microorganisms (Pieterse et al., 2016). The selection and alteration of the rhizosphere microbiome is one strategy for improving plant health and growth. The bacterial populations are most diverse and abundant in root-associated environments, they play important role by interacting with plant by mediating nutrient acquisition, regulating plant health and encountering the growth/activity of plant pathogens (Mazzola, 2004; Fabra et al., 2010; Raaijmakers and Mazzola, 2012). Likewise, fungi play an important role in determining soil functions (Scharl and Craven, 2003). A recent study demonstrated that endophytic fungi Phomopsis liquidambaris has ability to mitigate soil sickness by increasing the rhizosphere bacterial abundance and diversity with increased enzyme activities, soil respiration and carbon metabolism in long-term peanut monocropping system (Xie et al., 2020). It has been suggested that
fungal diversity may influence plant pathogen suppression and productivity (Wehner et al., 2010). Continuous cropping however, showed a considerably higher relative abundance of pathogenic fungi in rhizosphere soil than cropping rotation systems (Arafat et al., 2019; Liu et al., 2019a). In addition to bacteria and fungi, archaea as “third form of life” has been the hot topic. Many researchers have been interested in methanogenic archaea (Schink, 1992). A recent study reported the important archaeal population (methanogens), which seems likely to play role in upstream processes related to methane metabolism in rice (Liechty et al., 2020). In rhizosphere soil, the abundance and structure of archaeal communities of tomato plants differ, as they were shaped by soil type (Taffner et al., 2020). In the last two decades, the traditional microbiological approaches adopted, such as direct culture or molecular methods, have shown many limitations, because of unculturability of several important microbes in laboratory conditions (Kaeberlein et al., 2002; Kirk et al., 2004; Bloem et al., 2005; Sørensen et al., 2009). At present, at least 90% of these microbes are uncharacterized and remain un-cultured, representing an enormous reservoir of unexplored genetic and metabolic diversity. The developing sequencing techniques have been a powerful tool to understand complex microbial communities in broader and deeper prospective, providing access to many new species, genes, or novel molecules, contributing to variable applications, especially for biotechnology and agriculture (Streit and Schmitz, 2004; Tringe et al., 2005; Mocali and Benedetti, 2010).

Peanut (Arachis hypogaea L.) is one of the most important economic oil crops. It is a rich source of protein, vitamins, minerals and medicinally active components, such as flavonoids and phenolic compounds (Sui et al., 2018). According to the National Bureau of Statistics of China, peanut crop is being grown on area 4,608 km² during 2017 planting season (The National Bureau of Statistics of China, 2018) (Chen et al., 2020). Due to limited arable land and increasing pressure of regional agro-industrialization, large scale peanut monocropping is a common practice in China. Peanut has been moncropped for even 10 to 20 years in some fields of China’s leading peanut producing regions including Shandong and Henan (Chen et al., 2016). Continuous cultivation systems are bottlenecks, cause decline in crop production and limit agricultural sustainability. This phenomenon called soil sickness or negative plant-soil feedback (Huang et al., 2013). Legumes are particularly susceptible to this phenomenon, which is a major obstacle to sustainable crop productivity (Li et al., 2014a). The negative plant-soil feedback of continuously peanut cropping, caused by the imbalanced microbial populations has traditionally been attributed to the secretion of root exudates under continuous monocropping (Li et al., 2013; Li et al., 2014a). According to recent research, peanut roots under long-term monoculture consistently release the similar forms of exudates over a long period of time (Li et al., 2014a). These exudates may sometimes serve as a powerful screening element in the selection of rhizosphere microbiome. For example, during leguminous nodulation, flavonoids, a large class of phenylpropanoids found in root exudates, are known to attract rhizobia (Li et al., 2008). Exposure to similar exudation profiles over planting seasons can therefore enrich for some taxa while discriminating against others in continuous monocropping (Li et al., 2019b). The peanut rhizosphere microbiota is often selected at the cost of beneficial microbes that support peanut (Li et al., 2014a). In addition, the persistent secretion of a single type of root exudate may promote the development of soil-borne pathogens (Li et al., 2013), which in turn, causes soil sickness and inhibits peanut growth. Considerable dynamic changes in soil eukaryotic microorganisms have been found during continuous monocropping, especially the increase in pathogenic fungi and decrease in beneficial fungi (Arafat et al., 2019; Li et al., 2020a). Studies have shown that fungi, especially plant pathogenic fungi accumulated, and bacteria population decreased greatly in soil under continuous peanut cropping (Chen et al., 2012; 2014b). Peanut productivity gradually declined and severe diseases caused by Leptosphaerulina australis, Fusarium oxysporum, and Phoma sp. increased in consecutive monoculture, primarily because fungal pathogens increased with decreasing beneficial fungi in the continuous cropping soil (Li et al., 2014b). Moreover, bacterial populations were negatively selected in peanut continuous cropped soil, in contrast, a gradual decline of beneficial communities were recorded, which ultimately lead to reduced peanut yield (Chen et al., 2014b).
The focus of few available reports is mainly on either bacteria or fungi, where each community reported separately with no information about the archaeal microbiome. Nevertheless, the effect of peanut monocropping on bacterial, fungal and archaeal populations under continuous cropping cycles in a single study is not reported yet. We hypothesized that three years of continuous peanut monocropping imposed selection of microbiome by peanut plants reducing rhizosphere microbial diversity. To test this hypothesis, a greenhouse experiment for 3-years (2011 to 2013) under different continuous peanut monocropping cycles was performed. Culture-independent high throughput pyrosequencing-based approach was used to analyse microbial populations in the rhizosphere of peanut. This includes bacterial and archaeal 16S rRNA and fungal 18S rRNA genes. The aim of the present study was to elucidate the abundance and diversity of the bacterial, fungal and archaeal community structures using (16S,18S rDNA) pyrosequencing-based sequencing approach in peanut monocropping cycles for three consecutive years. Findings of this study should help to elucidate specific soil microbes that may impact soil functioning and ultimately effect plant growth.

**Materials and Methods**

*Management description and soil sampling*

The study was conducted during the three consecutive years from 2011 to 2013 at the research station of Shandong Academy of Agricultural Sciences, Jinan, Shandong, China (latitude 36°40′N; longitude 117°00′E). The soil used in this study comprised of 30% clay, 40% loam, and 30% sand, with no prior history of peanut cropping. Soil was divided into four parts and denoted as T1 or (cycle one), T2 or (cycle two), T3 or (cycle three), and Treatment B (unplanted soil). The peanut variety ‘Huayu22’, which is an important peanut variety of the region was planted from 2011 to 2013, hereafter as T1. The same peanut variety was planted between 2012 and 2013 and named as T2, while it was planted in 2013 and hereafter named as T3. The seeds of ‘Huayu22’ variety used throughout the experiments were obtained from the same batch and preserved in the standard laboratory conditions. Each year the plants were grown in the month of April and harvested in October, for the rest of the period (November to March), soil was left shallow/unplanted. Two seedlings were planted in each pot having diameter of (24 cm wide and 45 cm height), each treatment had 5 pots and a total of 20 pots, however only three pots were randomly taken from each treatment with uniform seedlings at the time of sampling and triplicate samples from each treatment were used for subsequent sequencing analyses. The peanut seedlings were irrigated using sterile water, and no fertilizer was applied during plant growth. Plants were uprooted at the pod-maturing stage and samples from the soil tightly attached to the roots of peanut plants (rhizosphere) were removed according to the method reported previously (Chen et al., 2012). The soil without planting (bulk soil) was designated as sample B. Bulk soil was also collected with three replicates in 2013. A total of 12 samples obtained for DNA extraction and sequencing from the four treatments. The obtained soil samples were put in labelled sterile bags and maintained at a low temperature on ice cooler box before being returned to the laboratory. DNA extraction was done on next day morning, metagenomic DNA samples were then stored at -80 until further analysis.

*DNA extraction, PCR amplification and 454 pyrosequencing*

Total DNA was extracted from rhizosphere soil from each sample using the PowerSoil™ DNA Isolation Kit (Mobio, USA) according to the instructions provided by the manufacturer. PCR amplification and high-throughput sequencing were performed at the National Human Genome Center (Shanghai, China). The 18s rDNA V4 region of fungi was amplified using the primers 3NDF and V4_euk_R1, V4_euk_R2 and V4_euk_R3, generating products varying from 450 to 500 bp in length. The bacterial 16s rRNA sequences were amplified by the primers 343F and 798R, generating products varying from 400 to 500 bp in length. Meanwhile, the archaeal 16s rRNA region was amplified using the primers A344F and A915R, and the length of fragments ranged from 525 to 575 bp. The primers sequences are presented in Supplementary Table-7. A
preliminary round of PCR amplification was carried out without the adaptors or sample-specific barcodes. Each 25 ul PCR reaction comprised, 10 ng of DNA and was performed using the FastStart High Fidelity PCR System (Roche Applied Science, Mannheim, Germany). Each reaction contained 2.75 ul FastStart 10 x reaction buffer, 1.8 mM MgCl2, 0.2 mM dNTP mix, 0.4 M of each primer, and 2 U FastStart HiFi polymerase. The cycling conditions were as follows: initial denaturation at 94 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C for 1 minute, annealing at 53 °C for 1 minute, and extension at 72 °C for 1 minute; a final extension phase was done at 72 °C for 10 minutes. PCR products were subjected to electrophoresis on 2% agarose gel for detection, the PCR products were then purified using the Axygen Gel Extraction Kit (Axygen, Union City, CA, USA). In a second round of PCR, the amplified fragments were added to the adaptors and then sequenced in the ROCHE 454 GS-FLX platform.

Sequence analysis
The raw sequences obtained from the four types of treatments were processed with the online tool Quantitative Insights Into Microbial Ecology (QIIME v 1.9.1) using default parameters. The sequences were first treated by removing short sequences (<200 bp), low average-quality score (<20) sequences, mismatching more than two bases of sequence and sequences that were not a perfect match to the barcode and primer sequence. Additionally, Chimera Slayer implemented in Mothur was employed to identify potential chimeric sequences. The sequences of fungi, bacteria, and archaea were clustered at 97% similarity identity using UCLUST (version 1.2.22). Operational taxonomic units (OTU), which were analyzed by distance according to the method reported by Li et al. (2014b), were filtered by the 0.005% of the sequence number that were sequenced as the threshold. The most abundant sequence from each OTU was the representative sequence for that OTU (Rampelotto et al., 2013). The Shannon diversity index and abundance of each sample were calculated at a 97% similarity cutoff for each taxonomic level (phylum, class, order, family, and genus). The final sequences were aligned from the chosen database: fungal 18S, and bacterial and archaeal 16S SILVA database (Release 119, http://www.arb-silva.de). The species were annotated using the RDP Classifier (version 2.2, http://sourceforge.net/projects/rdp-classifier/) at 0.8 as the confident threshold. Mitochondria, chloroplast sequences were removed by taxonomy-based filtering of non-targeted DNA sequences using exclude-seqs within the QIIME pipeline. The highest abundances of OTU sequences were selected for multiple sequence alignment and constructing the phylogenetic tree, using PyNAST (version 1.2.2, http://biocore.github.io/pynast) for bacterial and archaea analysis, while ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2) for fungi clustering.

Data analysis
Statistical analysis and visualizations were performed through online tool MicrobiomeAnalyst (Chong et al., 2020) and R software (https://www.r-project.org/). Data normalization was executed by rarifying the each OTU table at minimum library size, and low count OTUs that were present in < 10% samples were filtered, data was then normalized at total sum scaling (TSS) within MicrobiomeAnalyst pipeline. Statistical significance between groups was performed with Kruskal Wallis test (alpha diversity) and Permutational multivariate analysis of variance, PREMONOVA 999 Premutation (beta diversity). Venn diagrams were generated through online tool Jvenn (Bardou et al., 2014). Linear discriminant analysis (LDA) effect size (LeSe) method (Fisher, 1936) with score more than 2 and p-value < 0.05 was applied to estimate the effect size of each differentially abundant OTUs.

Results
In our preliminary experiments of investigating peanut growth, we have observed peanut productivity negatively affected by continuous cropping. Peanut production was decreased by 19.8% during the second
continuous cropping cycle (T2 in current study), and 33.4% during the third cycle (T3 in current study) (unpublished data). Therefore, we hypothesized that such reduction in the productivity could be the result of unbalanced below-ground microbial populations in the peanut rhizosphere caused by consecutive monocropping. To investigate the peanut microbiomes, metagenome analyses were pursued. A total of twelve samples from three years continuous monocropping cycles of peanut rhizosphere and unplanted soils (bulk soil) were processed. After quality control and removing short reads, a total of 110,412 read with an average length of 440 bp for bacterial dataset, 75,437 total reads with an average length of 452 bp for fungal dataset and 106,921 total reads with an average length of 540 bp for archaea dataset were obtained (Supplementary Table 1). Each dataset was rarefied to a minimum number of reads to avoid sample heterogenicity (Supplementary Figure 1). The OTU tables containing 14226 OTUs for bacteria, 2620 OTUs for fungi and 4321 OTUs for archaea datasets are represented in (Supplementary Tables 2-4).

Alpha diversity was measured using Shannon index, Observed OTUs, ACE, Chao, Simpson and Coverage for the respective three datasets, and the results are summarized in (Supplementary Table 5). Our results show that alpha diversity decreased as the continuous monocropping cycles proceeds. For both bacterial and archael samples, the highest alpha diversity (Shannon Index) was observed in the T1 (initial peanut monocropping cycle in 2011-2013), which then decreased gradually in T2 and T3 (2nd cycle in 2012-2013 and third cycle in 2013, respectively), while the least alpha diversity was observed in the B treatment (bulk soil with no peanut plantation) (Figure 1a and c).

![Figure 1](image-url)

**Figure 1.** Alpha and beta diversity of peanut rhizosphere microbiome under consecutive cropping cycles (a-f).

Shannon index based (richness and evenness) of bacteria (a), fungi (b) and archaea (c) in peanut rhizosphere soil samples. Beta diversity of bacterial (d), fungal (e) and archael microbiomes (f). Each group of triplicate samples is indicated by one colour. The square with orange colour indicates the B treatment or soil with no plantation, grey indicate the T2 treatment, green indicate T3 treatment. Statistical significance for alpha diversity (a-c) was performed with Kruskal Wallis test and Permutational multivariate analysis of variance, PREMONOVA for beta diversity (d-f), p < 0.05 was considered significant between groups.
Similar trends were observed for fungal community, except least alpha diversity observed in T2 and T3 treatments compared to B treatment (Figure 1b). Simultaneously, beta diversity results from distance matrices for the Jaccard indices sets using constrained principal component analysis (CPCA) revealed that, consecutive cropping cycles were the factor of key shift in the bacterial, fungal and archaeal community differentiation. Analysis of these communities separately for bacteria only, fungi only and archaea only exhibited that each microbial community was affected by the consecutive monocropping cycles (Figure 1). Samples of T1 and B treatments were clustered separately from the T2, T3 treatments in almost all populations studied (Figure 1d, e, f), however, less clear clustering observed for T2 and T3 treatments. This was particularly true for the fungi and archaeal groups. The differences were statistically significant when assessed for both bacterial p < 0.001 and Fungal community p < 0.003, except for the archaea p < 0.079.

To better understand the monocropping effect on microbial populations under different consecutive cropping cycles, we determined the microbiomes shared or unique to each group through Venn diagrams (Figure 2). Looking at the bacterial samples, a total of 1025 OTUs were shared by all the groups regardless of treatments, comprising 7% of the total OTUs in the dataset, and majority of these belonging to bacterial communities, Acidimicrobiales, Actinomycetales, Rhizobiales, Rhodobacterales, Sphingomonadales (Figure 2a). Only 497 OTUs exhibited group-specificity, which is 3% of all OTUs. T2 treatment samples have the most types 171 OTUs that were not present in other treatments, those OTUs mainly belong to Actinomycetales, Bacillales, Burkholderiales and Gaiellales. Moreover, the total number of bacterial OTUs in the rhizosphere soil groups T1 (3454), T2 (3756), T3 (3262) were much higher compared to the unplanted soil, B (2953) (Figure 2a). Simultaneously, a total of 1878 OTUs were shared by the different groups, accounting for 20% of all OTUs in the fungal dataset, and most of them were member of Agaricomycetes, Ambisporaceae, Atractiellomycetes, and Eurotiumycetes (Figure 2b). The number of Unique OTUs in each group T1, T2, and T3 were 12, 10 and 32 respectively, and exists <1% of all OTUs in each group. We also found 30 abundant OTUs that were present only in unplanted soil B (Figure 2b). As for the archaeal community, 434 OTUs were shared all together by different groups, accounting for 8% of the total OTUs and majority of these core community belongs to Candidatus_Nitrososphaera, and Methanomassiliicoccaceae (Figure 2c). Merely, 290 OTUs that is 5% of total OTUs exhibited the group-specificity, and those OTUs were predominantly dominated by Nitrososphaeraceae family.

Figure 2. Venn diagram representation of peanut rhizosphere microbiome (a-c) Representation of unique and shared OTUs between different groups of (a) bacterial (b) fungal and (c) archaeal communities under three years of consecutive monocropping cycles. Small bar graphs showing the total number of OTUs in each group.

Moreover, the presence and abundance of various phyla and order were assessed through taxonomically assigning all abundant sequences with the RDP classification. A total of 20 phyla for bacteria, 5 phyla for fungi and 4 phyla for archaea were detected through RDP taxonomy (Figure 3). The top bacterial phyla of...
Actinobacteria (39.46%), Proteobacteria (39.30%), Gemmatimonadetes (6.71%) and Bacteroidetes (5.65%), together contributed more than (>91%) of the total bacterial diversity. Both Proteobacteria and Actinobacteria were overrepresented in B treatment (soil with no plantation), while relatively overrepresented in T3 compared to other treatments (Figure 3a). In case of fungi, among the five phyla detected, the Ascomycota alone accounting for (>86%) of the total fungal diversity, followed by Basidiomycota (5.25%), Basal fungal lineages (4.66%) and Glomeromycota (3.44%), while Edhazardia was detected in low abundance. The relative abundances of major fungal phyla, Ascomycota and Basidiomycota increased under continuous cropping cycles (Figure 3b). For instance, Ascomycota were highly abundant in T2 treatment, while least abundance was observed in early cycle T1 treatment (Figure 3b). Likewise, decreased abundance of Basidiomycota was observed in T2 treatment, nonetheless it dramatically increased in T3 treatment. Unlike bacteria, only four phyla were detected for archaeal community across all samples. Archaeal OTUs were mainly assigned to Crenarchaeota as the top most phylum (80.58%), followed by Euryarchaeota (17.43%) as the second most abundant phylum (Figure 3b). Together these two phyla constitute >97% of total archaeal diversity. Other phyla such as Parvarchaeota was present in relatively low abundance (0.04%) while a small proportion of reads (0.04%) were remained unknown. The abundance of Crenarchaeota increased with the consecutive cropping years, it was overrepresented in T3 and B treatment (unplanted soil), compare to early cropping cycles T1 and T2 treatments (Figure 3b). Euryarchaeota on the other hand, was abundant in early cycles and showed gradual decrease from T1 to T3 treatment as the monocropping proceeds for the consecutive cycles.

Figure 3. Microbiome communities at the phylum level found in peanut rhizosphere under continuous cropping cycles as treatments (a, b, c). Bacterial microbiome communities from 16S amplicon sequencing (a). Fungal microbiome communities from ITS amplicon sequencing (b). Archaeal microbiome communities from 16S amplicon sequencing (c). Each color in the left ribbon circle represents a phylum and the right panel denotes the samples. The outer rings show the composition of each phylum or each sample, by sample type or vice versa.

A comparative analysis of taxonomic assignments at order level between treatments for top ten bacterial, fungal and archaeal populations are presented in (Figure 4). Actinomycetales was the top most abundant bacterial order presented equivalently across all samples of rhizosphere soils, T1 (21.16%), T2 (21.47%), T3 (21.12%), it was relatively high abundant in unplanted soil B (22.46%) (Figure 4a). Decreasing trends were observed for Rhodospirillales and Sphingomonadales under continuous cycles, as both were depleted from T1 (8.84%), (4.79%), T2 (7.96%), (6.48%), and T3 treatment (9.92%), (5.83%), compare to B treatment (10.09%), (10.64%) respectively. Likewise, Rhizobiales was increased under T3 (6.34%) and T2 treatments (6.22%), though it was less abundant in T1 (5.17%) and in B (4.35%). In contrast, compare to unplanted soil B (4.94%), the relative abundance of Gaiellales was higher in first monocropping cycle T1 (5.73%), it was depleted in later cropping cycles both in T2 (3.95%), and in T3 (4.44%). This was also true for Burkholderiales as it showed decreasing abundance from T1>T2>T3>B. (Figure 4a). For fungal population, Sordariomycetes as the top most order was overrepresented in unplanted soil B (38.48%) than that in the rhizosphere soil
surrounded by peanut root in T1 (32.36%), T2 (31.63%) and T3 (25.77%) (Figure 4b). Conversely, peanut rhizosphere soil harbor Phoma in high abundance in T1 (18.62%), T2 (33.07%) and T3 (24.31%) compare to the B or unplanted soil (4.99%). Mucorales and Agaricomycetes (9.50%) and (12.19%) respectively were overrepresented in T3. Pezizomycetes was overrepresented in B (9.99%) and T1 (2.00%), while Glomeraceae was relatively abundant in T1 (3.40%) and T2 (3.43%), compare to T3 and unplanted soil B (1.08%) and (1.29%) respectively (Figure 4b). As for the archaea community at order level, Nitrososphaerales (80.53%) was the most abundant order across all the samples. The abundance of this order increased in the third monocropping cycle, T3 (85.87%) compare to first and second cropping cycles, T1 (72.46%) and T2 (77.35%) treatments; It was abundant in B (86.46%) (unplanted soil) (Figure 4c). Interestingly, E2 member of Thermoplasmata (17.15%) which was the second most abundant order within archaeal community showed decreased trends under consecutive monocropping. The abundance was higher in both T1 (25.37%) and T2 (20.31%), which then decreased in T3 (12.73) treatment. The least abundance of this order was observed in unplanted soil, B (10.18%). Other order such as YLA114 was present in more or less similar amount regardless of treatments, T1 (1.82%), T2 (1.88%) and T3 (1.08%), while, it was present in relatively higher abundance in B samples (3.06%) (Figure 4c).

The OTU tables of three corresponding datasets were used to further analyse and classify the treatment-specific biomarkers through the LetSe approach. A total of 150 OTUs for bacteria, 19 OTUs for fungi and 87 OTUs for archaea were found significantly changed under consecutive cropping cycles as treatments. The list of significant OTUs is presented in (Supplementary Table 6). The relative abundance of some of these biomarkers for bacteria, fungi and archaea are shown in (Figure 5). These OTUs including OUT-1620 (Solirubrobacterales), OUT-1692 (Cytophagaceae) were enriched in T1 treatment, OUT-2032 (Ramlbacter), OUT-1454 (Gemmamimonadetes) enriched in T2, OUT-1039 (Skemanella), OUT-1128 (Solirubrobacterales) were enriched in T3, and OUT-532 (Steroidobacter), OUT-1383 (spinghomonas) in B treatment showed differential abundance patterns for bacteria (Figure 5a). Similarly, fungal OTUs such as, OUT-2111 (Eurotiomycetes), OUT-733 (Atractiellomycetes) were highly abundant in T1, OUT-2158 (Eurotiomycetes), OUT-1446 (Phoma) in T2, OUT-121 (Ambisporaceae), OUT-425 (Atractiellomycetes) in T3 and OUT-1748 (Glomeraceae), OUT-787 (Atractiellomycetes) in B were highly abundant (Figure 5b). Likewise, some exemplar OTUs of archaea including, OTU839 (Desulfurococcales), OTU1431 (Thermoplasmata) were overrepresented in T1, OTU856 (Desulfurococcales), OTU1197 (YLA114) in T2, OTU557 (Desulfurococcales), OTU448 (Candidatus_Nitrososphaera) overrepresented in T3 and OTU674...
(Desulfurococcales), OTU1103 (Candidatus_Nitrososphaera) were enriched in unplanted soil B but showed depleted patterns in other treatments (Figure 5c).

**Figure 5.** Treatment-specific significant OTUs under peanut continuous cropping cycles were identified using the linear discriminant analysis (LDA) effect size (LefSe) approach. A list of significant OTUs is accessible through Supplementary Data. This figure indicates relative abundance of certain exemplar biomarkers for bacteria (a), fungi (b), and archaea (c).

**Discussion**

The phenomenon of soil sickness or negative soil-plant feedback caused by continuous cropping is traditionally regarded to change in the soil microbial populations. Continuous peanut cropping has been proven to greatly effect soil microbial communities (Chen *et al.*, 2020; Li *et al.*, 2020b). In a previous study, it was shown that the composition of peanut microbiome was shifted during three years of continuous peanut monocropping (Chen *et al.*, 2014a). In our greenhouse experiments observing peanut growth, it appeared that peanut productivity was negatively affected by continuous cropping, it decreased during the second and third continuous cropping cycles, T2 and T3 in current study, (unpublished data). We hypothesized that consecutive peanut monocropping may have imposed selection of specific below-ground microbial taxa by altering the microbial diversity in the rhizosphere. Our results from pyrosequencing (available sequencing platform at that time) support our hypothesis and provided insights into the bacterial, fungal and archaeal community compositions, abundance and revealed remarkable differences among the soil microorganism communities under three years of different peanut cropping regimes.

Our results showed that, microbial diversity in peanut rhizosphere was gradually decreased as the continuous cropping cycle proceeds (Figure 1). Similar trends were observed in previously reported study, where long-term monocropping reduced the diversity and altered the abundance and structure of peanut rhizosphere microbiome (Chen *et al.*, 2020). Our study, however, in contrast with the study reported by the same group, where phylotype diversity of fungi increased over the time of continuous cropping (Chen *et al.*, 2012). The contrasting results might be due the effect of plant growth period, as the samples were taken at...
seedling and flowering stages of peanut growth during monocropping. Yet, the successional progression of certain microbial phylotypes, as well as the interaction mechanisms between microorganisms and plants, require additional investigation.

Results of constrained principal component analysis (CPCA) showed that microbial communities associated with monocropped peanut rhizospheres varied substantially from those in soil without peanut plantation and between different cropping cycles (Figure 1d, e, f). Samples of B treatments and T1, each grouped separately from the other T2, T3 treatments in most cases. This indicated that the duration of the monocropping had obvious effect on both fungal and archaeal population structure. There were statistically significant differences between these groups under monocropping cycles. Our findings mirror the previous outcomes where fungal composition varied significantly under continuous peanut monocropping systems (Chen et al., 2020).

In our study, the richness (number of OTUs) of bacterial population was higher in the rhizosphere soil (T1, T2, T3) than that in the unplanted soil B (Figure 2a). This is explainable, and seems likely because of the so-called rhizosphere effect, the soil around the roots (rhizosphere) come to contact with root exudates and serve as food web for many microbes to be attracted and colonized. In contrast, unplanted soil is a steady environment with less nutrients for the microbes to prey on. Previously it was reported that assembly of the rhizosphere microbiota was distinct from that of unplanted soil (Lopes et al., 2021). Despite being grown the same peanut genotype ('Huayu22') for all the three years monocropping cycles, some of the OTUs in T1, T2, and T3 rhizosphere communities generated in this project show little to no overlap in their predominant community members (Figure 2a, b, c). Therefore, the communities observed here do not reflect continuum, but rather a distinct population of varying degrees of preference for a particular niche over time. Perhaps due to the specific secretion of root exudates under continuous monocropping (Li et al., 2013; Li et al., 2014a). According to a recent research, peanut roots under long-term monocropping routinely discharge the same types of exudates over a long period of time (Li et al., 2014a). Hence, exposure to similar exudation profiles over long period can enrich some specific taxa over others (Li et al., 2019b).

Although the relative abundances of major bacterial phyla were different between the soil and rhizosphere samples or even among the different cropping cycles from T1 to T3, Actinobacteria, Proteobacteria, and Gemmatimonadetes were the dominant phyla across the peanut rhizosphere soil (Figure 3). This was in agreement with a previous study, where it was reported that Actinobacteria and Proteobacteria were dominant in peanut rhizosphere (Dai et al., 2019), employing that these phyla are the common bacterial populations of the peanut rhizosphere soil. Interestingly, the composition of the bacterial population at the phylum and order level did not change drastically over the years of monocropping cycles in our study, however, the structure of the fungal community did change between different groups with distinct dominant taxa. With regard to fungal phyla, Ascomycota, and Basidiomycota were the most dominant phyla in our samples (Figure 3). This concurs with that of previous research in which peanut monocropping altered the composition of fungal communities under long term monocropping (Chen et al., 2020), where Ascomycota, and Basidiomycota were highly abundant; however, our results differ from their study in such a way that the phylum Zygomycota, was not present in our samples. This is explainable, perhaps because of genotype effect as they used different plant genotypes in their study. In our study, archaeal phyla Crenarchaeota and Euryarchaeota were found to be the major contributors of their respective populations in peanut rhizosphere (Figure 3). Previously, metagenomic study of archaeal community demonstrated that phylum Crenarchaeota and Euryarchaeota were the dominant phylum present on different niches such as soil and leaf of arugula plant (Eruca sativa Mill.) (Taffner et al., 2019).

Furthermore, the relative abundance of many bacterial orders showed enriched and depleted patterns across all samples (rhizosphere and unplanted soil). This was particularly true for the continuous monocropping cycles (T1, T2 and T3) in our study. For instance, Actinomycetales as a major bacterial order was less abundance in rhizosphere samples but relatively high abundant in unplanted soil B (Figure 4a). Rhodospirillales, Sphingomonadales and Burkholderiales showed decreased tendency from T1 to T3 under
monocropping cycles. In contrast, Rhizobiales displayed increased abundance in later monocropping cycles T3 and T2 treatments, it was less abundant in T1 and fallow soil. Gaiellales which was less abundant in all the libraries also showed decreasing trends in later cropping cycles both in T2 and in T3 (Figure 4a). Previously, these bacterial communities including Actinomycetales, Sphingomonadales, Rhizobiales, Burkholderiales and Gaiellales all were reported with increased and/or decreased tendency under continuous peanut cropping cycles (Chen et al., 2014a). The majority of the taxa at the order level in the reported study had decreased abundance and diversity that was regarded to consecutive monocropping, with only a few taxa with increased abundance and diversity over time. It would be interesting to further reveal what possible functional roles of this changing microbiome could play under consecutive monocropping.

For fungal population, although Sordariomycetes as the top most order dominated both type of soils (rhizosphere and unplanted soil), it was exclusively presented in B than that in the soil surrounded by peanut roots. In rhizosphere its abundance gradually decreased with the passing years under monocropping from T1>T2>T3 (Figure 4b). Sordariomycetes play distinct roles in the plant-soil feedback, the highest abundance of this order was previously detected in bulk soil compare to rhizosphere soil under continuous cropping in tobacco (Jiang et al., 2020). Many Phoma species are plant fungal pathogens. Previously, it was shown that compare to control soil, Phoma species displayed higher relative abundances in fields where peanut has been monocultured consecutively (Li et al., 2014a; Li et al., 2014b). In our study, the known pathogenic fungi Phoma was highly abundant in peanut rhizosphere with increasing tendency under continuous monocropping from early to later years. One of the most important aspects of soil microbial diversity is AMF species diversity, which helps plants absorb nutrients, improves soil structure, and regulates the global carbon and nitrogen cycle. Glomeraceae is group of mycorrhizal arbuscular fungi, which form symbiotic (Mycorrhizal) relationship with plant roots (Pan et al., 2021). In our study, Although Glomeraceae was dominant in rhizosphere soil, its relative abundance decreased in later planting cycle T3 compare to first two cycles (T1, T2). The decreasing abundance of this beneficial fungi perhaps is the direct effect of continuous monocropping. Other fungal taxa such as Mucorales and Agaricomycetes both showed elevated tendency in later planting cycle T3, while Pezizomycetes was overrepresented in B and T1 (Figure 4b).

We found Nitrososphaerales as the most abundant order which increased at the third monocropping cycle, T3 compare to first and second cropping cycles, and comparatively abundant in unplanted soil (Figure 4c). Nitrososphaerales are known as soil ammonia oxidizers, which transform NH₃ to NO₂ and play a significant role in N cycling in terrestrial ecosystems. A significant increase in the average relative abundance of Nitrososphaerales clad was reported under long term greenhouse monocropping (Liu et al., 2019b). The diversity of Nitrososphaerales was also reported from natural ecosystems and agricultural soil, where it was overrepresented in wet land and soil from tea plantations (Lynn et al., 2017). Thermoplasmata have a mixotrophic lifestyle that involves CO₂ fixation as well as formaldehyde and acetate assimilation, and they have important potential functions in the carbon, sulfur, and arsenic processes (Hu et al., 2020). Thermoplasmata in our study showed decreased trends under consecutive monocropping, the abundance was higher in both T1 and T2, which then decreased in T3 (Figure 4c).

Additionally, several important treatment-specific OTUs belonging to these orders and genera level showed differential abundance patterns based on LEfSe analysis (Figure 5a). In our study, bacterial exemplar OTUs belonging to Solirubrobacterales, Cytophagaceae, Ralibacter, Gemmatimonadetes, Skermanella, Solirubrobacterales, Steroidobacter, and spinghomonas were screened out as potential biomarkers. Previously these taxa were shown to be affected by different agronomic practices such as long term monocropping, chemical fertilizer applications, crop rotation systems and even plant development age (Chen et al., 2014a; Huang et al., 2017; Lynn et al., 2017; Tang et al., 2020). Similarly, fungal OTUs belonging to Eurotiomycetes, Atractiellomycetes, Ambisporaceae, Glomeraceae and Phoma were differently abundant (Figure 5b). Phoma species are known as fungal pathogens and the increased abundance of this fungi were reported under monocropping (Li et al., 2014a; Li et al., 2014b).
Some exemplar biomarkers of archaea including, Desulfurococcales, Thermoplasmata, YLA114, Candidatus nitrososphaera showed enriched and depleted patterns under continuous monocropping (Figure 5c). Candidatus nitrososphaera has been identified as a potentially essential taxon in the upper (10 cm) layer of soil under natural forming conditions (Liao et al., 2019). Previously, Desulfurococcales was reported as the dominant groups of non-acidic soils in the hot springs from different geographical locations (Song et al., 2013). Taken together, we have shown the taxonomic profiles of peanut rhizosphere communities that were affected by continuous peanut monocropping. Continuous peanut cultivation led to a substantial decrease in soil microbial abundance and diversity as the monocropping time proceeds. Several bacterial, fungal and archaeal taxa were significantly changed in abundance under continuous peanut cultivation. Pathogenic fungi were increased and beneficial fungal taxa decreased under continuous monocropping. Our findings suggests that, management of rhizosphere microbes and preserving the equilibrium between beneficial and harmful microbes in the soil are critical to the success of cropping practices.

Conclusions

In this study, we have shown that fungal, bacterial, and archaeal communities exhibited different responses to continuous peanut cropping. Fungal diversity increased with peanut monoculture: pathogenic fungi increased and beneficial fungi decreased with the continuous cropping. The bacterial diversity and abundance decreased, except for in the third cropping cycle. The archaeal diversity (beta diversity) was not significantly different among the control and monoculture soil, whereas archaeal abundance was affected by increased peanut continuous cropping. Findings of this study should help to elucidate specific soil microbes that may impact soil functioning and ultimately effect plant growth.

Authors' Contributions

Conceptualization, H.C; Data curation, A.I.M, Y.S, X.Z; Formal analysis, A.I.M, Y.S and X.Z; Methodology, SF; Software, A.I.M, X.Z; Supervision, H.C and A. I. M; Validation, A.I.M, X.Z; Writing – original draft, H.C and A. I. M; Writing – review and editing, A. I. M. and H.C All authors read and approved the final manuscript.

Data Deposit
Raw reads of all the 16S bacterial, archaeal and 18S fungal amplicon sequences reported in this study have been deposited into the National Center for Biotechnology Information BioProject database under accession number of PRJNA349110.

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Conflict of Interests
The authors declare that there are no conflicts of interest related to this article.
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