# Effects of Continuous Cropping on Bacterial Community Structure in Rhizospheric Soil of Sweet Potato

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# Abstract

**Background:** Continuous cropping obstacles from sweet potato are widespread, which seriously reduce the yield and quality, restrict the sustainable development of sweet potato industry. Bacteria are the most abundant in rhizospheric soil and have a certain relationship with continuous cropping obstacles. However, there are few reports on how continuous cropping affected the bacterial community structure in the rhizospheric soil of sweet potato. In this study, high-throughput sequencing technique was used to explore the changes of rhizospheric soil bacterial community structure of different sweet potato varieties, and the correlation between soil characteristics and this bacterial community after continuous cropping, so as to provide a theoretical basis for the prevention and control of sweet potato continuous cropping obstacles.

**Results:** After two years of continuous cropping, the results showed that (1) the dominant bacteria phlya in rhizospheric soils from both Xushu18 and Yizi138 were Proteobacteria, Acidobacteria, and Actinobacteria. The most dominant genus was Subgroup 6_norank. Significant changes in the relative abundance of rhizospheric soil bacteria were observed for two sweet potato varieties. (2) Bacterial richness and diversity indexes of rhizospheric soil from Xushu18 were higher than those from Yizi138 after continuous cropping. Moreover, the beneficial *Lysobacter* and *Bacillus* were more prevalent in Xushu18, but Yizi138 contained more harmful Gemmatimonadetes. (3) Soil pH decreased after continuous cropping, and redundancy analysis showed that soil pH was significantly correlated with bacterial community. Spearman's rank correlations coefficients analysis demonstrated that pH was positively correlated with Planctomycetes and Acidobacteria, and negatively correlated with Actinobacteria and Firmicutes.

**Conclusions:** After continuous cropping of sweet potato, the bacterial community structure and physicochemical properties in the rhizospheric soil were unbalanced, and the changes of different sweet potato varieties were different. The contents of *Lysobacter* and *Bacillus* were higher in the sweet potato variety resistant to continuous cropping. It provides a basis for the development of special microbial fertilizer for sweet potatoes to alleviate continuous cropping obstacle.

# Background

The sweet potato [*Ipomoea batatas* (L.) Lam.] of the Family Convolvulaceae is a major food crop cultivated worldwide and widely used. It is a high yield and efficiency crop, resistant to drought and barrenness, and has strong adaptability[1-3]. In addition, sweet potato is rich in nutrients and has cancer preventing properties, so it become more and more popular [4]. As China adjusted its planting structure and implemented underground water pressure mining policies in some areas, sweet potato planting area is increasing yearly. Due to the limited arable land in China, sweet potatoes are grown in the same field continuously. However, continuous cropping obstacles are common with sweet potato, which can lead to reduced yield and quality, severe plant death or even failure to harvest [5-6]. In recent years, the

phenomenon of continuous cropping obstacles of sweet potato has become serious, and it has become a bottleneck problem that restricts the sustainable development of sweet potato industry in China[7].

Many factors are related to continuous cropping obstacles, such as soil enzyme activity, soil physical and chemical properties, root exudates, and soil microbial community[8-9]. Soil microorganisms are the key factors related to changes in soil quality, fertility and productivity, and play an important role in ecosystem [10-12]. The rhizosphere is the narrow region of soil that surrounds plant roots, where plants, soil, and microorganisms interact dynamically [13]. Rhizospheric soil microorganisms are closely related to the absorption and transformation of soil nutrients. Therefore, their community structure is an important factor affecting plant growth, development, and health [14-17]. Many reports indicate that continuous cropping changes the microbial structure of rhizospheric soil [18-21]. These changes have further led to severe continuous cropping obstacles[9]. Therefore, the relationship between rhizospheric soil microbial community structure and continuous cropping obstacles has become one of the research hotspots[7].

Bacteria are the most abundant group of rhizospheric soil microorganisms and important component[22]. Increasing reports have noted the changes in bacterial community structure in rhizospheric soil that are caused by continuous cropping. Wu et al.[23] and Wu et al. [24] studied the changes in bacterial community structure in the rhizospheric soil of konjac and vanilla after continuous cropping, and found that continuous cropping changed the composition and structure of bacterial communities, resulting in a reduction in beneficial bacteria and an increase in harmful bacteria. Tan et al. [25] used high-throughput sequencing to study changes in the diversity and composition of rhizospheric soil microbes and endophytes of *Panax notoginseng* after three years of continuous cropping, and found that bacteria decreased over time. Further, the bacterial diversity of the rhizospheric soil of healthy *Panax notoginseng* was greater than that of diseased plants. At the same time, CCA analysis found that P, pH, and soil organic matter have the greatest impact on bacterial community composition. In addition, the results of Na et al. [26] revealed that the number of bacterial species and the α-diversity of bacterial communities in the rhizospheric soil of *Lycium barbarum* L. decreased significantly after continuous cropping, which provided a theoretical basis for analyzing the mechanisms underlying continuous cropping obstacles.

The detrimental effects of continuous cropping on rhizospheric soil microbial community structures are becoming elicited based on studies on different crops. However, studies focusing on the continuous cropping of sweet potato were mainly concentrated with the prevention and treatment of pests and diseases[27-28]. Accordingly, little was known about how this process affects the rhizospheric soil bacterial community structure. For the first time, we studied the effects of continuous cropping on bacterial community structure in the rhizospheric soil of two sweet potato varieties (Xushu18 that was resistant to continuous cropping and Yizi 138 that was susceptible to continuous cropping) using high-throughput technique, as well as the correlation between soil characteristics and this bacterial community after continuous cropping. This was performed to explore how continuous cropping affects bacterial community structure in rhizospheric soil of two sweet potato varieties, and to provide a theoretical basis for the application of biological control continuous cropping obstacles associated with sweet potato.

# Methods

## Description of the study area and materials

The experimental site was located in the Dishang test station of Institute of Cereal and Oil Grops, Hebei Academy of Agriculture and Forestry Sciences, Hebei Province, China (37°56'24.62" N, 114°42'46.96" E). The average temperature was 12.5 °C. The mean annual precipitation was 494 mm [7]. This region has a warm temperate sub - humid continental monsoon climate and the local soil type is Mottlic Hapli-Ustic Argosols (cinnamon soil). Two sweet potato varieties were used as experimental materials, one is Xushu 18 (X18) that is resistant to continuous cropping and the other is Yizi 138 (Y138) that is susceptible to continuous cropping. Both Xushu 18 and Yizi 138 were collected from the Institute of Cereal and Oil Crops, Hebei Academy of Agriculture and Forestry Sciences (Shijiazhuang, China).

## Experimental design and sample collection

Our experiment began in 2015. Sweet potato seedlings were planted on May 6 and harvested on October 7 each year, and were continuously planted for two years. After each harvest, the fields were left fallow for the rest of the year. A random block design was used, with three experimental repetitions. The ridge distance was 85 cm, seedling spacing was 25 cm for five rows, length of the plot was 6 m, and the area of the plot was 25.5 $m^2$ [7]. The agronomic management was the same for all fields in two years. Rhizospheric soil was collected after 30 days of planting and before 7 days of harvest respectively from 2015 to 2016, a total of four sampling periods. The rhizospheric soil that closely adhered to the root of sweet potato was taken according to the method of Sun et al. [51]. Each sweet potato variety at each sampling period, the rhizospheric soils of 15 sweet potatoes that were located in an "S" shape were collected from each plot and mixed thoroughly to generate one composite soil sample per plot, All samples were put into separate sterile plastic bags, placed in an ice box, and quickly taken to the laboratory. Each samples were passed through a 2-mm sieve, and then divided the composite soil sample into two portion. One portion was stored at −80 °C for microbial analysis, and the other portion was air-dried for soil characteristics analysis.

## The soil physicochemical properties analysis

Soil pH was determined using the electrode method (2.5:1 water : soil ratio), and the available Ca, B, Fe, Mn, and Zn were measured using the atomic absorption method according to Du and Gao [52].

## DNA extraction, PCR amplification, and Illumina MiSeq sequencing

The DNA was extracted from the samples using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to manufacturer's protocols. The V4 - V5 regions of the bacteria 16S rRNA gene were amplified by PCR (95°C for 2 min, followed by 25 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s and a final extension at 72°C for 5 min) using primers 515F 5'-barcode-GTGCCAGCMGCCGCGG)-3' and 907R 5'-CCGTCAATTCMTTTRAGTTT-3', where the barcode was an eight-base sequence unique to each

sample. The PCR reactions were performed in triplicate 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA. The extraction and purification of amplicons were performed according to the report by Yang et al.[34]. Purified PCR products were quantified using Qubit®3.0 (Life Invitrogen) and every 24 amplicons whose barcodes were different were mixed equally. The pooled DNA product was used to construct Illumina Pair-End library following Illumina's genomic DNA library preparation procedure. Then the amplicon library was paired-end sequenced (2 × 250) on an Illumina MiSeq platform (Shanghai BIOZERON Co., Ltd.) according to the standard protocols[7]. Complete data sets generated in this study were deposited in the NCBI Sequence Read Archive database under accession number SRP214716.

## Processing and statistical analysis of sequencing data

In order to obtain high quality sequences, quality control and sequence quality filtering were applied according to Yang et al.[34] to remove low quality sequences. Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE￼version 7.1 http://drive5.com/uparse/) and chimeric sequences were identified and removed using UCHIME. The phylogenetic affiliation of each 16S rRNA gene sequence was analyzed using RDP Classifier (http://rdp.cme.msu.edu/) against the SILVA (http://www.arb-silva.de) 16S rRNA database with a confidence threshold of 70% [34].

The Chao richness index, Shannon and Simpson diversity indexes were generated using Mothur v.1.21.1 [53]. Principal component analysis (PCA) was performed using R-forge (the Vegan 2.0 package was used to generate a PCA figure). The shared and unique OTUs were analyzed using Venn diagrams by VennDiagram. Redundancy analysis (RDA) which was employed to explore the relationship between environmental factors and bacterial communities, Spearman's rank correlations coefficients ,and Heatmap figures were performed using Vegan packages in R. The data of the physicochemical properties of rhizospheric soil were analyzed using one-way analysis of variance (ANOVA) ($P < 0.05$) through SPSS v21.0[34].

# Results

## The rhizospheric soil physicochemical properties of sweet potato

After sweet potato continuous cropping two years, the content of available Mn in the rhizospheric soil of X18 and Y138 respectively decreased by 32.68% and 31.14% at the early stage of planting , and decreased by27.35% and 31.10% at the early stage of harvest. (Table 1). The soil pH of X18 and Y138 respectively decreased by 2.72% and 3.11% at the early stage of planting, the change was not significant at the early stage of harvest. The content of available Ca in the rhizospheric soil of X18 and Y138 respectively increased by29.80% and 38.97% , available Zn increased by 56.11% and 43.19% at the early stage of planting, and available Ca respectively increased by 30.75% and 26.47%, available Zn increased by 29.46% and 30.81% at the early stage of harvest. Available Fe of X18 and Y138 respectively decreased by 18.61% and 17.08% at the early stage of harvest, the change was not significant at the early stage of

planting. The content of available B in the rhizospheric soil of X18 decreased by 20.63% at the early stage of planting, while the change of Y138 was not significant.

## α-diversity of bacteria in rhizospheric soil of sweet potato

The average coverage of all samples was 96.19% (**Table 2**). The rarefaction curve of each sample had already approached a saturation plateau (**Fig. 1**), which indicated that the sequencing had reached saturation, and the results were thought to truly reflect the sample conditions. The reads of per sample ranged from 24695 to 37688. The OTUs of per sample ranged from 3137 to 3734. Richness index (Chao) and diversity index (Shannon and Simpson) of X18 and Y138 were calculated based on the number of bacterial OTUs. Unlike the Shannon index, the larger the Simpson index value, the lower the community diversity. The Chao and Shannon index values of X18 and Y138 were higher at the early stage of harvest than that at the early stage of planting, and they were the opposite of the Simpson index, indicating that species richness and diversity of the two communities at the early stage of harvest were higher. At the same time, the Chao and Shannon indexes of X18 were higher than those of Y138, which were contrary to the Simpson index. In other words, the richness and diversity of bacteria in the rhizospheric soil of X18 were higher than those of Y138.

**Table 1.** The physicochemical properties of different soil samples

| Sample | Ca (g kg$^{-1}$) | B (mg kg$^{-1}$) | Fe (mg kg$^{-1}$) | Mn (mg kg$^{-1}$) | Zn (mg kg$^{-1}$) | pH |
|---|---|---|---|---|---|---|
| X18-1 | 0.34±0.01c | 0.77±0.07a | 4.64±0.67c | 2.60±0.28a | 1.65±0.13d | 8.57±0.06a |
| X18-2 | 0.35±0.01c | 0.71±0.08ab | 5.86±0.30a | 2.54±0.06a | 1.96±0.01c | 8.60±0.00a |
| X18-3 | 0.45±0.01b | 0.61±0.06b | 4.78±0.16bc | 1.75±0.07b | 2.58±0.09a | 8.33±0.06b |
| X18-4 | 0.45±0.00ab | 0.63±0.05b | 4.77±0.01bc | 1.85±0.05b | 2.54±0.02a | 8.50±0.10a |
| Y138-1 | 0.34±0.02c | 0.78±0.05a | 4.52±0.24c | 2.56±0.01a | 1.73±0.06d | 8.57±0.06a |
| Y138-2 | 0.36±0.01c | 0.69±0.04ab | 5.31±0.53ab | 2.71±0.26a | 1.66±0.17d | 8.60±0.00a |
| Y138-3 | 0.47±0.02a | 0.69±0.08ab | 4.70±0.18bc | 1.76±0.12b | 2.48±0.00a | 8.30±0.00b |
| Y138-4 | 0.46±0.01ab | 0.72±0.09ab | 4.41±0.03c | 1.87±0.04b | 2.17±0.03b | 8.53±0.06a |

Values are mean±standard deviation of triplicate determinations. Different letters in the same column indicate significant differences of the same sweet potato variaties at different sampling times at a level of P<0.05 using Duncan's multiple range tests; Ca:available calcium; B: available boron; Fe: available iron; Mn : available Manganese; Zn: available zinc. X18: Xushu 18; Y138:Yizi 138; 1 and 2 represent sampling of early planting and early harvest in 2015; 3 and 4 represent sampling of early planting and early harvest in 2016, respectively.

**Table 2.** MiSeq sequencing results and α-diversity index of sweet potato rhizospheric soil samples

| Sample ID | Reads | 0.97 | | | | |
|---|---|---|---|---|---|---|
| | | OTU | Chao | Coverage | Shannon | Simpson |
| X18-1 | 37688 | 3326 | 4626 (4440, 4842) | 0.969407 | 6.69 (6.68, 6.71) | 0.0033 (0.0032, 0.0034) |
| X18-2 | 28047 | 3405 | 4955 (4743, 5201) | 0.954327 | 6.91 (6.89, 6.93) | 0.0028 (0.0027, 0.0029) |
| X18-3 | 35422 | 3207 | 4509 (4324, 4724) | 0.967139 | 6.67 (6.65, 6.68) | 0.0034 (0.0033, 0.0035) |
| X18-4 | 33236 | 3734 | 5250 (5046, 5485) | 0.960434 | 7.02 (7.01, 7.04) | 0.0023 (0.0022, 0.0023) |
| Y138-1 | 32513 | 3137 | 4388 (4209, 4596) | 0.964876 | 6.64 (6.62, 6.66) | 0.0036 (0.0035, 0.0037) |
| Y138-2 | 24695 | 3230 | 4808 (4590, 5061) | 0.948654 | 6.89 (6.87, 6.91) | 0.0028 (0.0027, 0.0029) |
| Y138-3 | 35866 | 3156 | 4511 (4316, 4739) | 0.968187 | 6.75 (6.74, 6.77) | 0.0028 (0.0028, 0.0029) |
| Y138-4 | 33436 | 3602 | 5074 (4873, 5307) | 0.961897 | 6.97 (6.95, 6.98) | 0.0024 (0.0024, 0.0025) |

X18: Xushu 18; Y138:Yizi 138; 1 and 2 represent sampling of early planting and early harvest in 2015, 3 and 4 represent sampling of early planting and early harvest in 2016, respectively; OTU: operational taxonomic unit; The numbers within parentheses are the lower and upper limits in statistics of the corresponding date, respectively.

## Community composition analysis of bacteria in rhizospheric soil of sweet potato

At the phylum level (**Fig. 2**), X18 and Y138 rhizospheric soil bacteria mainly belonged to Proteobacteria (28.5%-34.9%), Acidobacteria (10.4%-21.1%), Actinobacteria (11.3%-18.1%), Planctomycetes (5.2%-9.9%), Chloroflexi (4.6%-9.1%), Bacteroidetes (3.4%-6.1%), Gemmatimonadetes (3.0%-7.4%), and Firmicutes (1.4%-10.9%). Among them, Proteobacteria was the most prevalent, and Acidobacteria and Actinobacteria were next.

After sweet potato continuous cropping two years, the content of Proteobacteria in the rhizospheric soil of X18 and Y138 decreased by 17.30% and 8.05% at the early stage of harvest, respectively. Acidobacteria in the rhizospheric soil of X18 and Y138 showed a decreasing trend and finally increased slightly, while Actinobacteria showed the opposite trend. The content of Firmicutes in the rhizospheric soil of X18 and Y138 was higher at the early stage of planting than that at the early stage of harvest. while the change of Planctomycetes was opposite. Further, the content of Chloroflexi and Gemmatimonadetes in the rhizospheric soil of X18 and Y138 showed an increasing trend, Chloroflexi respectively increased by 81.09% and 96.69%, and Gemmatimonadetes increased by 103.11% and 122.56%, respectively. In addition, the content of Gemmatimonadetes in the rhizospheric soil of Y138 was higher than that of X18, especially in 2016.

At the genus level (**Fig. 3**), the relative abundance of Subgroup 6_norank (6.59% - 14.74%), *Nitrosomonadaceae*_uncultured (1.83%-6.40%), *Anaerolineaceae*_uncultured (1.75%-3.63%) were the top three dominant bacteria genus in all rhizospheric soils of X18 and Y138, other major genus included *Bacillus* (0.65%-4.14%), MSB-1E8_norank (0.87%-3.83%), *Tepidisphaeraceae*_norank (1.71%-2.56% ), *Xanthomonadales*_norank (0.62%-2.08%), and *Lysobacter* (0.55%-2.06%). After two years of continuous cropping, the content of Subgroup 6_norank in the rhizospheric soil of X18 and Y138 showed a decreasing trend, respectively decreased by 54.34% and 52.66%, and then increased slightly at the early stage of harvest in 2016. However, *Nitrosomonadaceae*-uncultured and *Anaerolineaceae*-uncultured in the rhizospheric soil of X18 and Y138 were present at low levels at the early stage of planting but increased at the early stage of harvest. while *Bacillus* and *Lysobacter* showed the opposite trend. Moreover, in every sampling period, the content of *Lysobacter* in the rhizospheric soil of X18 was higher than that of Y138. *Bacillus* was the same as *Lysobacter,* except for the early stage of planting in 2015. In addition, In the second year of continuous cropping, the reduction of *Lysobacter* in the rhizospheric soil of X18 and Y138 was 1.3 times and 2.4 times of the reduction in the first year.

## Venn analysis of bacteria in rhizospheric soil of sweet potato

Venn diagrams directly showed the overlapped and unique OTUs of all samples. (**Fig. 4**). After two years of continuous cropping, the number of OTUs shared by all samples were 507. In the four sampling periods, there were 95, 158, 127, and 202 unique OTUs in the rhizospheric soils of X18. However, the unique OTUs in the rhizospheric soils of Y138 were 89, 124,141, and 159, respectively. With the increase in continuous cropping time, the number of OTUs specific to X18 and Y138 showed an increasing trend. The number of OTUs specific to X18 was more than that of Y138 (except for the early stage of planting in 2016), indicating that continuous cropping led to changes in the rhizospheric soil bacterial communities of X18 and Y138. Further, the differences were largest during the early harvest period of 2016.

## Heatmap and clustering analysis and PCA of bacteria in rhizospheric soil of sweet potato

Heatmap and clustering analysis results for the 40 phyla from the different samples were shown in **Fig. 5**. Based on the relative abundance value of the heatmap and color changes, the difference in bacteria composition in the rhizospheric soil of X18 and Y138 could be seen more clearly. Furthermore, the

clustering results showed that all samples grouped into two large clusters and the samples of the same continuous cropping years were gathered together. In addition, X18 and Y138 from the same sampling period grouped together.

The OTUs of X18 and Y138 were subjected to PCA. The extracted two principal components explained 72.48% of the variation in total (**Fig. 6**). With an increase in continuous cropping years, the distribution of X18 samples at different sampling times was relatively discrete. A similar trend was observed for Y138. It indicated that bacterial community structure in rhizospheric soil of X18 and Y138 changed with the extension of continuous cropping time. However, in the same sampling period, X18 and Y138 samples were relatively close to each other. With the increase in continuous cropping time, the distance between these samples also gradually increased, which indicated that differences between their bacterial communities were also increasingly large. These results were consistent with the results of heatmap and cluster analysis in **Fig. 5**. Overall, these results indicated that (i) continuous cropping resulted in the changes of bacterial community structure in rhizospheric soil of X18 and Y138; (ii) bacterial community compositions in rhizospheric soil of X18 and Y138 were relatively similar in the same sampling period.

## Relationship between dominant bacterialphyla and rhizospheric soil properties of sweet potato

RDA performed on the top 10 bacterial phyla and rhizospheric soil properties of X18 and Y138 showed that the first and second RDA components explained 56.57% and 28.05% of the total variation, respectively (**Fig. 7**). The effect of soil properties on bacterial community structure was found to occur in the following order: soil pH > Ca > Mn > Zn > B > Fe. To investigate the significances of the effects of soil environmental factors on bacterial community composition, we calculated the $r^2$ and Pr. The results showed that soil pH ($r^2$=0.9737, Pr=0.004), available Ca ($r^2$=0.8815, Pr=0.011) were significantly correlated with the bacterial community. It indicated that soil pH was a strong predictor of bacterial community in the rhizospheric soil of X18 and Y138.

Moreover, Spearman's correlation coefficient was used to evaluate the relationship between soil physicochemical properties and bacterial phyla abundance (**Fig. 8**). Results showed the following correlations between bacterial phyla and soil properties: pH was positively correlated with Planctomycetes (R = 0.97) and Acidobacteria (R = 0.93) and negatively correlated with Actinobacteria (R = −0.79) and Firmicutes (R = −0.72); available Ca was positively correlated with Actinobacteria (R=0.89) and Gemmatimonadetes (R=0.86), and negatively correlated with Acidobacteria (R = −0.79), Planctomycetes (R= −0.75) and Nitrospirae (R= −0.72); available Mn was positively correlated with Planctomycetes (R = 0.81) and Acidobacteria (R = 0.78), and negatively correlated with Gemmatimonadetes (R = −0.81) and Actinobacteria (R = −0.80). At the same time, it can be seen from the **Fig.8** that the soil physicochemical properties were divided into two groups, with available Ca and available Zn clustered into one group and the rest clustered into another, indicating that available Ca and available Zn had a similar effect on the bacteria but different from the rest.

# Discussion

In order to have a more comprehensive understanding of the bacterial community structure of sweet potato rhizospheric soil, Illumina Miseq high-throughput sequencing technology based on 16S rRNA gene was adopted in this study [29-30]. The V4 - V5 highly variable region of 16S rRNA gene was selected as the sequencing region because a previous study proved that this region was the best sequencing region among nine highly-variable regions of 16S rRNA gene fragments [31].

The most dominant phylum in the rhizospheric soil bacterial community after sweet potato continuous cropping was Proteobacteria, which was consistent with the results reported by Li et al.[8], Yin et al.[22] and Zhu et al.[32]. The main function of Proteobacteria was to decompose organic matter and promote plant growth [33-34]. The most dominant genus was Subgroup 6_norank, which was consistent with the report of Yin et al.[22]. Meanwhile, with an increase in continuous cropping years, the content of Proteobacteria in the rhizospheric soil of X18 and Y138 decreased, which was consistent with the report of Liu et al.[35]. Acidobacteria in the rhizospheric soil of X18 and Y138 showed a tendency to decrease gradually and then increase slightly. Actinobacteria in the rhizospheric soil of X18 and Y138 tended to increase gradually and then decrease slightly, which was almost consistent with the results reported by Yin et al. [22] and our previous study [36], but different from those reported by Li et al. [8] and Wu et al. [24]. These discrepancies might be due to differences in crop types, continuous cropping years, and soil types. It has been reported that the changes in the diversity of soil bacterial community species differed greatly based on different years of continuous cropping [37], and plant species, plant genotypes and soil types had a certain impact on the structure of the soil microbial community [13,38-39].

This study also found that some microbial changes in the rhizospheric soil of X18 and Y138 were affected by growth stage. For example, the content of Firmicutes was greater at the early stage of planting and decreased at the early harvest stage. This indicates that the growth of sweet potato has a certain influence on it. when the sweet potato was gone, its content recovered. In addition, these changes may also be related to the season, Li et al. [40] found that seasonal changes have a significant effect on soil microbes, but that different microbes vary with season.

After continuous cropping, the contend of *Lysobacter* in the rhizospheric soil of X18 and Y138 decreased, which is an important biocontrol bacteria with strong bacteriolytic and bacteriostatic effects [41]. For example, *Lysobacterenzymogenes* OH 11 is found to exert a significant bacteriostatic effect on soft rot pathogen of sweet potato. However, Gemmatimonadetes, a harmful bacteria which can lead to N loss and reduce crop growth [34], in the rhizospheric soil of sweet potato were found to increase.

These results suggested that the bacterial community structure was imbalanced after continuous cropping, which was consistent with previous reports [8,23-24]. These changes have further led to the continuous cropping obstacles of **X18 and Y138** increased. **In addition, the yield of X18 and Y138 decreased significantly after continuous cropping.** the average yield of X18 for two consecutive years was 12.36 t hm$^{-2}$ and 10.64 t hm$^{-2}$, respectively. Y138 was 9.15 t hm$^{-2}$ and 2.66 t hm$^{-2}$. The yield reduction of Y138 was significantly higher than that of X18.

Therefore, it is very important to maintain the balance of bacterial community structure to prevent and control continuous cropping obstacles. In actual production, ecological means can be used, such as the rational application of microbial fertilizer and soil modifier[42-44] to increase the content of beneficial microorganisms in the soil to alleviate continuous cropping obstacles.

The differences in rhizospheric soil bacterial community composition between the two sweet potato varieties after continuous cropping were found. With an increase in continuous cropping years, the number of OTUs specific to X18 increased more than those specific to Y138, and the community structure became increasingly different. According to the results of α-diversity analysis, the rhizospheric soil of X18 had higher bacteria richness and diversity than Y138; Thus, the community structure of X18 was relatively stable, the tolerance to soil environmental changes was greater, and the resistance to continuous cropping obstacles was stronger, whereas the community structure of Y138 was susceptible to continuous cropping. In addition, the content of *Lysobacter* and *Bacillus* in the X18 rhizospheric soil were higher than those in Y138. Many studies showed that *Bacillus* may resist some soil–borne diseases [13,24,34]. The more content of *Lysobacter* and *Bacillus* would be more conducive to the growth of X18. In contrast, the rhizospheric soil of Y138 contained more harmful Gemmatimonadetes. Therefore, it is also very important to choose the varieties of sweet potato that are resistant to continuous cropping in production practice.

After sweet potato continuous cropping, the rhizospheric soil physicochemical properties were also unbalanced. Moreover, it was found that soil pH had the greatest influence on bacterial community structure, which was consistent with the results of Fernández-Calviño and Bååth [45], Li et al.[8], and Qiao et al. [46]. Soil pH can affect soil microbial physiological metabolism and change the competitive relationship between microbial communities or inhibit the growth of non-adapted microbes[26], thus affecting microbial community structures. In terms of the three bacteria phyla with the most relative content, there was a positive correlation between Acidobacteria and pH, which was consistent with the results of Yang et al. [34]; Further, there was a negative correlation between Actinobacteria and pH, which was consistent with that reported by Alami et al.[47] and Wu et al. [24]. However, the results were different from that reported by Li et al. [8], which might have been caused by different plant age or genotype [48].

Similar to bacteria, fungi are important decomposers in soil. Research reports that certain fungal taxa might act as antagonists or pathogens of notoginseng under continuous cropping systems [49]. In addition, many fungi are closely related to plant diseases [50].We previously studied the effects of continuous cropping of sweet potato on the fungal community structure in rhizospheric soil [7]. The results showed that the fungi diversity and richness in rhizospheric soil of X18 and Y138 were significantly increased after continuous cropping; Furthermore, the content of Fusarium, the pathogen of sweet potato root rot, was increased. the fungal community structure of the sweet potato rhizospheric soil was unbalanced after continuous cropping.

Overall, both the bacteria and fungi community structure of the sweet potato rhizospheric soil changed after continuous cropping, and the microbial community structure is unbalanced, which might be an

important factor causing the continuous cropping obstacles of sweet potato. Thus, during actual production, attention should be paid to maintain the stability of sweet potato rhizospheric soil micro-ecology to relieve the continuous cropping obstacles. In addition, due to continuous cropping obstacles are related to many factors within the crop–soil– microbe system, such as soil enzyme activity and root exudates. It is necessary to combine with many other methods to solve this problem. Therefore, we will carry out further in-depth research in the future to provide a more comprehensive basis for the prevention and control of sweet potato continuous cropping obstacles.

# Conclusions

In conclusion, continuous cropping of sweet potato resulted in an imbalance in the bacterial community structure and physicochemical properties in the rhizospheric soil, and the changes of different sweet potato varieties were different. The contents of *Lysobacter* and *Bacillus* were higher in the sweet potato variety that resistant to continuous cropping. It provides a basis for the development of special microbial fertilizer for sweet potatoes. Therefore, further research is needed to see if *Lysobacter* and *Bacillus* could be used to produce microbial fertilizers to alleviate continuous cropping obstacles of sweet potato.

# Abbreviations

X18:xushu18;Y138:Yizi138; OTUs: Operational taxonomic units ; PCA: Principal component analysis; RDA: Redundancy analysis; ANOVA: one-way analysis of variance.

# Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Availability of data and materials

All data generated or analysed during this study are included in this published article. Sequence data of this study have been deposited in the NCBI Sequence Read Archive database under accession number SRP214716.

### Competing interests

The authors declare that they have no competing interests.

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## Authors' contributions

ZM and ZJG designed this experiment. LL and WJ performed samples collection. YH, JX, XW, ZT, RJ and ML executed the experiment. ZYG, MH and ZT analyzed the data. ZYG, YH and MH finished the manuscript. All authors read and approved the final manuscript.

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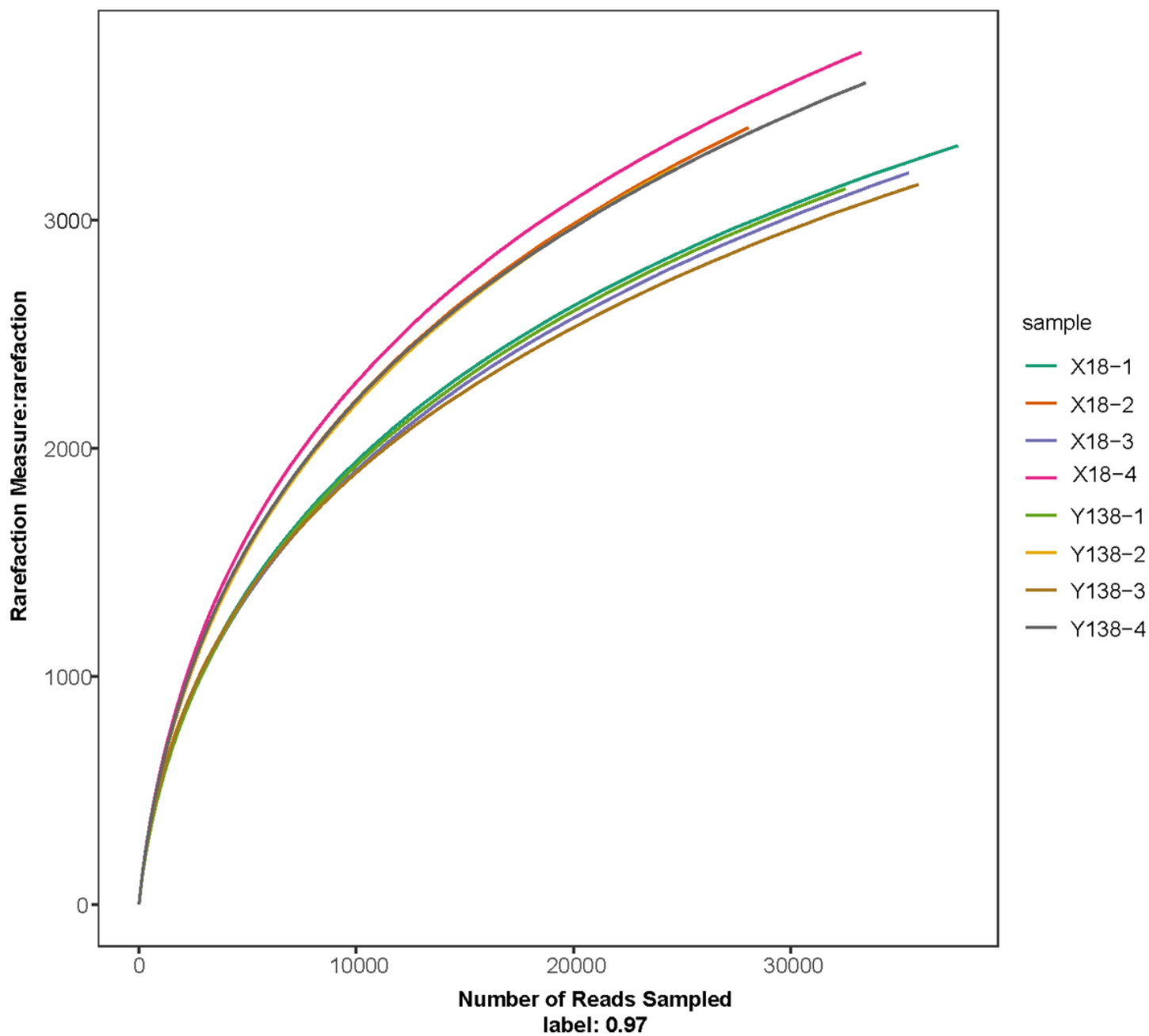
# Figures

**Figure 1**

Rarefaction curves of all soil samples. X18: Xushu 18; Y138: Yizi 138; 1 and 2 represent sampling of early planting and early harvest in 2015; 3 and 4 represent sampling of early planting and early harvest in 2016, respectively.
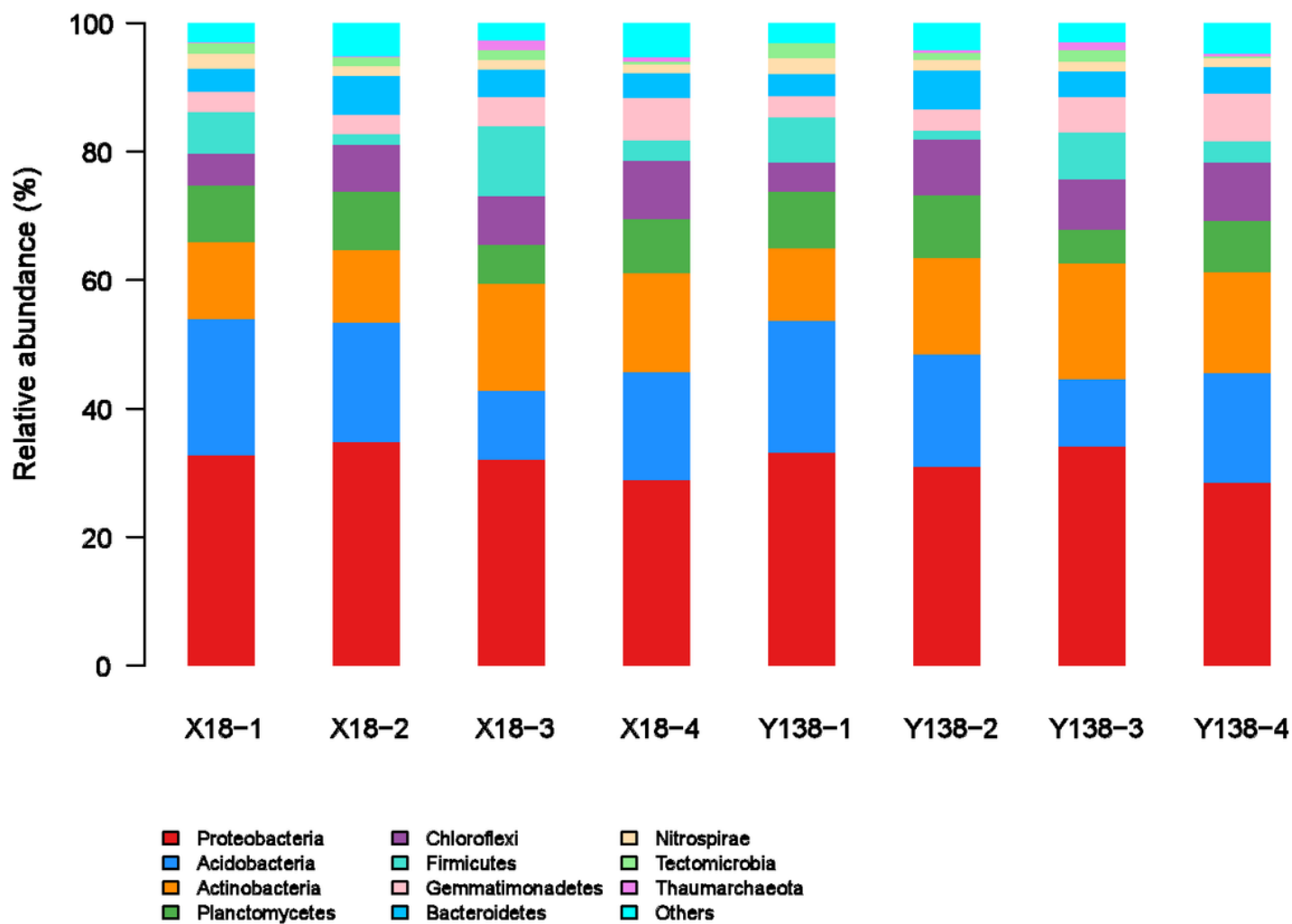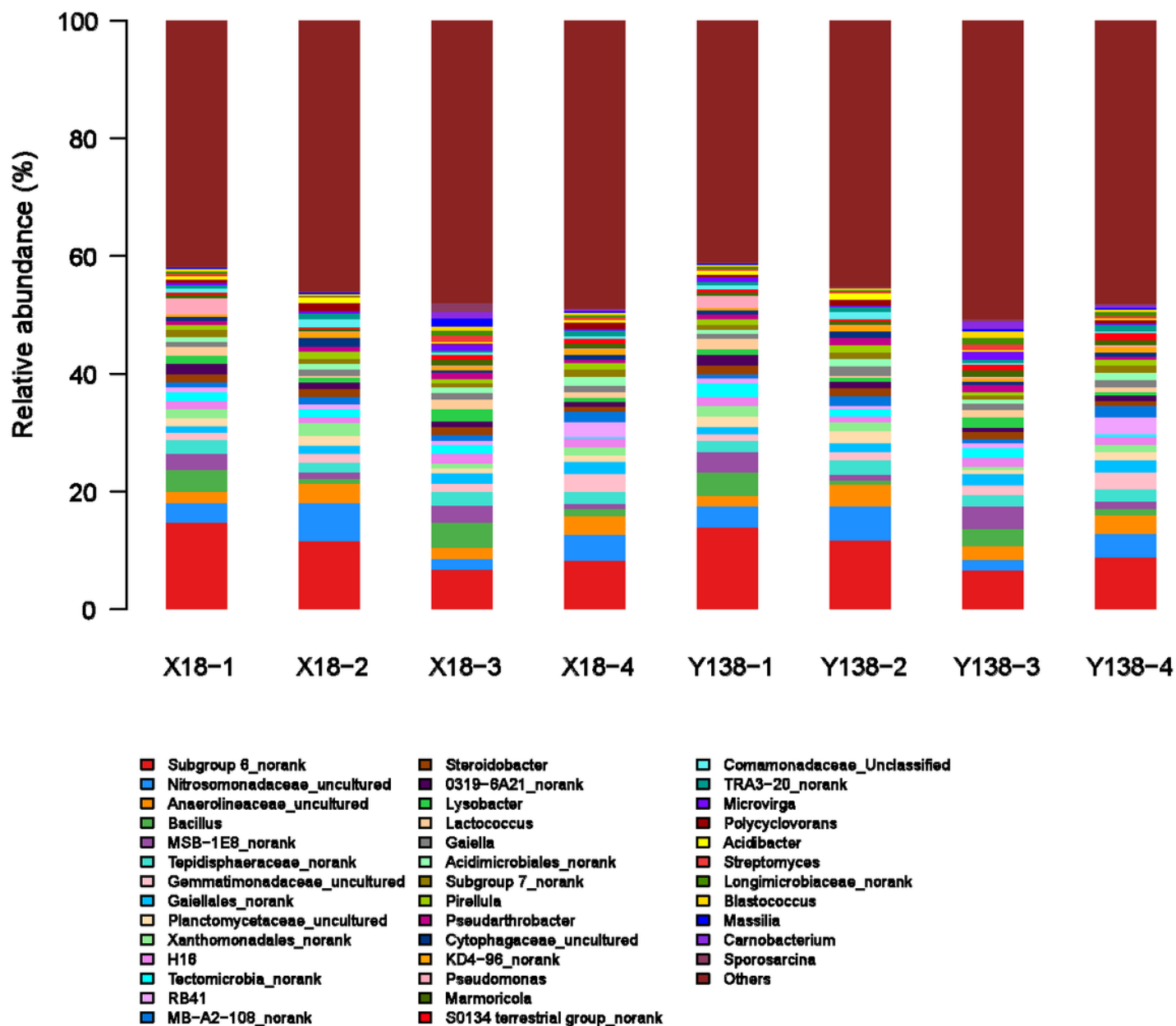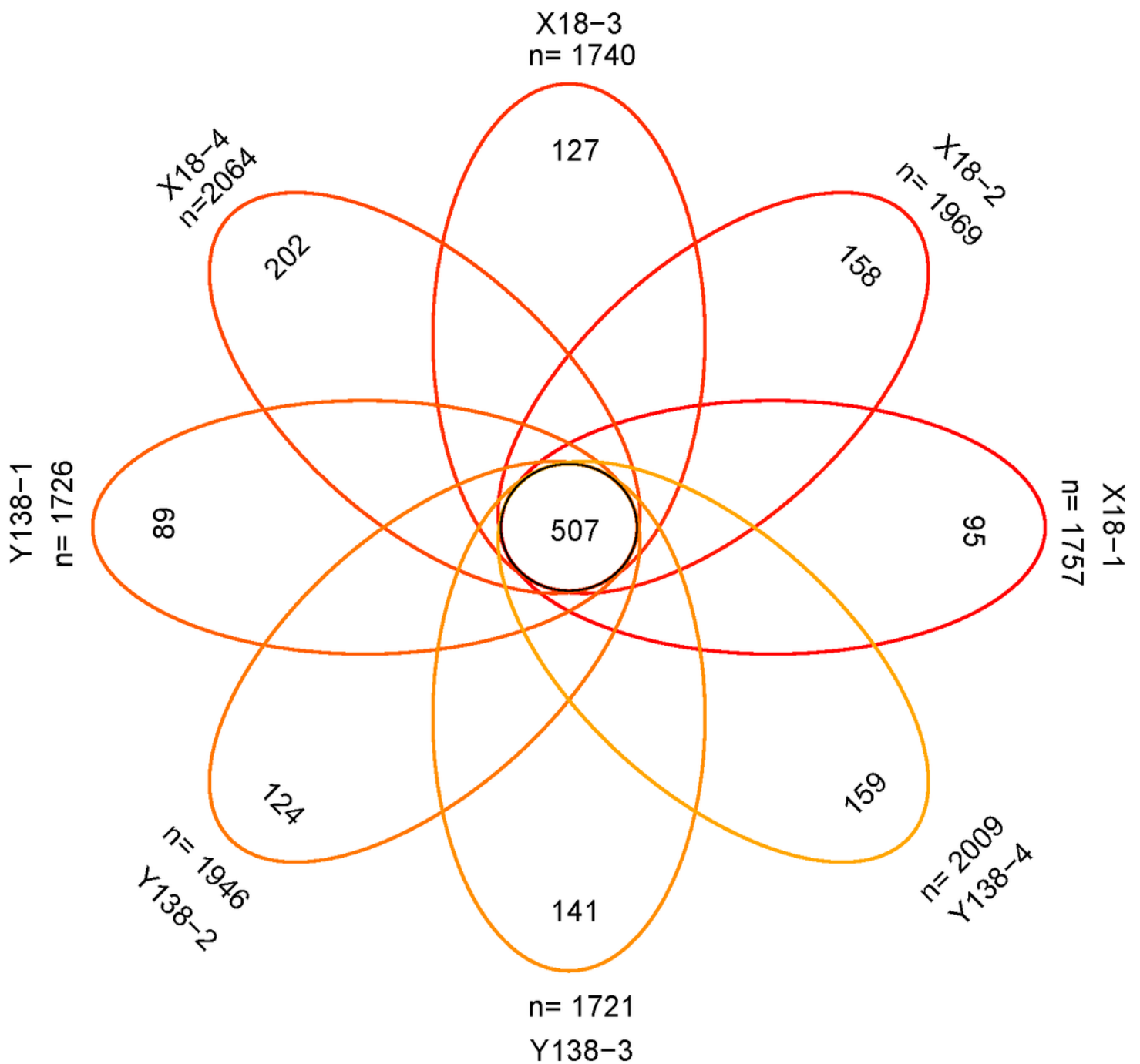
**Figure 2**

Relative abundance of bacterial phyla in the rhizospheric soil of continuous cropping sweet potato X18 and Y138. X18: Xushu 18; Y138:Yizi 138; 1 and 2 represent sampling of early planting and early harvest in 2015; 3 and 4 represent sampling of early planting and early harvest in 2016, respectively.

**Figure 3**

Relative abundance of bacterial genera in the rhizospheric soil of continuous cropping sweet potato X18 and Y138. X18: Xushu 18; Y138: Yizi 138; 1 and 2 represent sampling of early planting and early harvest in 2015; 3 and 4 represent sampling of early planting and early harvest in 2016, respectively.
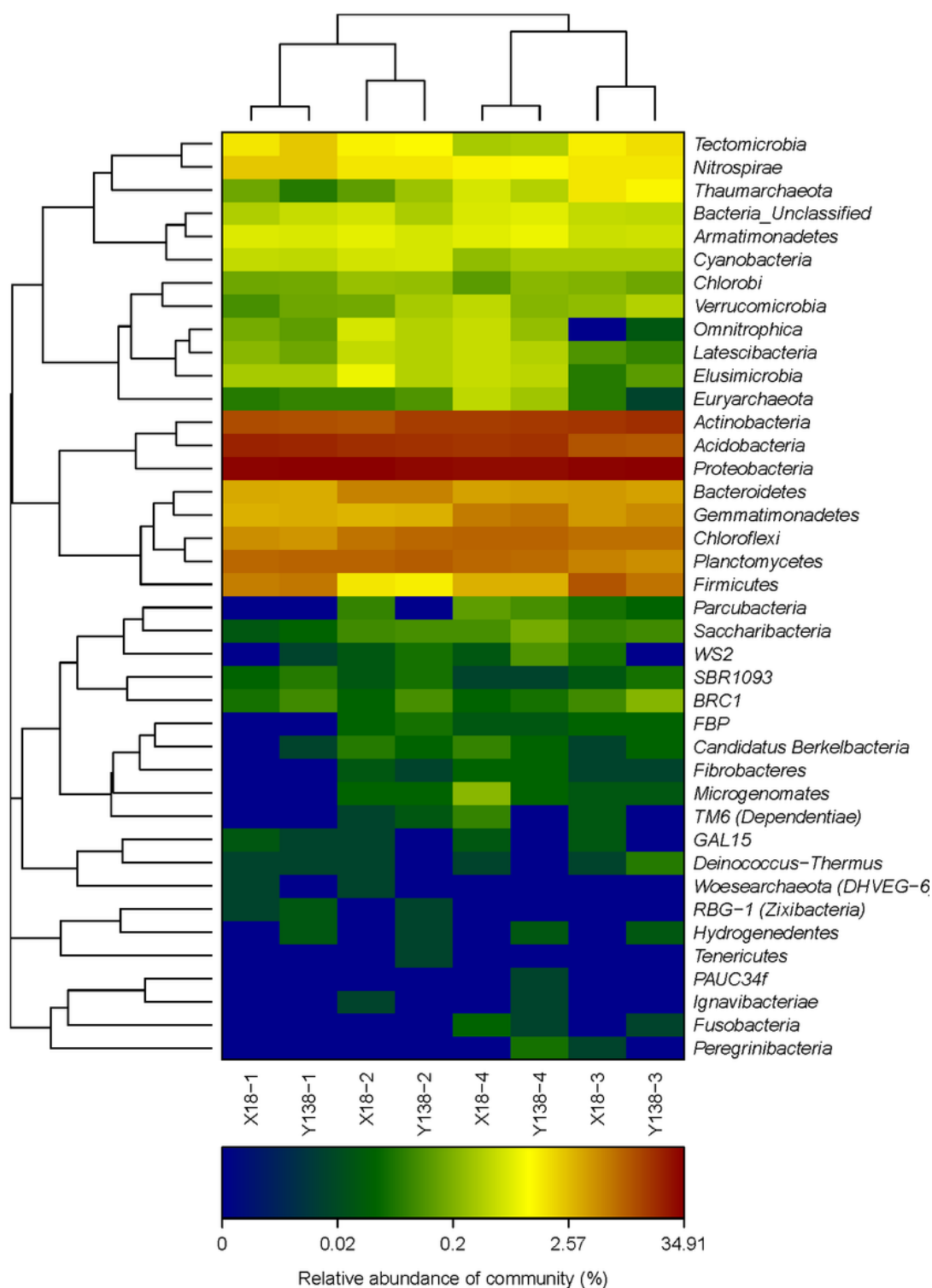
**Figure 4**

Number of common and unique operational taxonomic units (OTUs) based on Venn analysis. X18: Xushu 18; Y138:Yizi 138; 1 and 2 represent sampling of early planting and early harvest in 2015; 3 and 4 represent sampling of early planting and early harvest in 2016, respectively.

**Figure 5**

Microbial community heatmap and cluster analysis of the bacterial phyla detected across all samples. X18: Xushu 18; Y138: Yizi 138; 1 and 2 represent sampling of early planting and early harvest in 2015; 3 and 4 represent sampling of early planting and early harvest in 2016, respectively. The relative values for bacterial phyla are indicated by color intensity with the legend at the bottom of the picture.
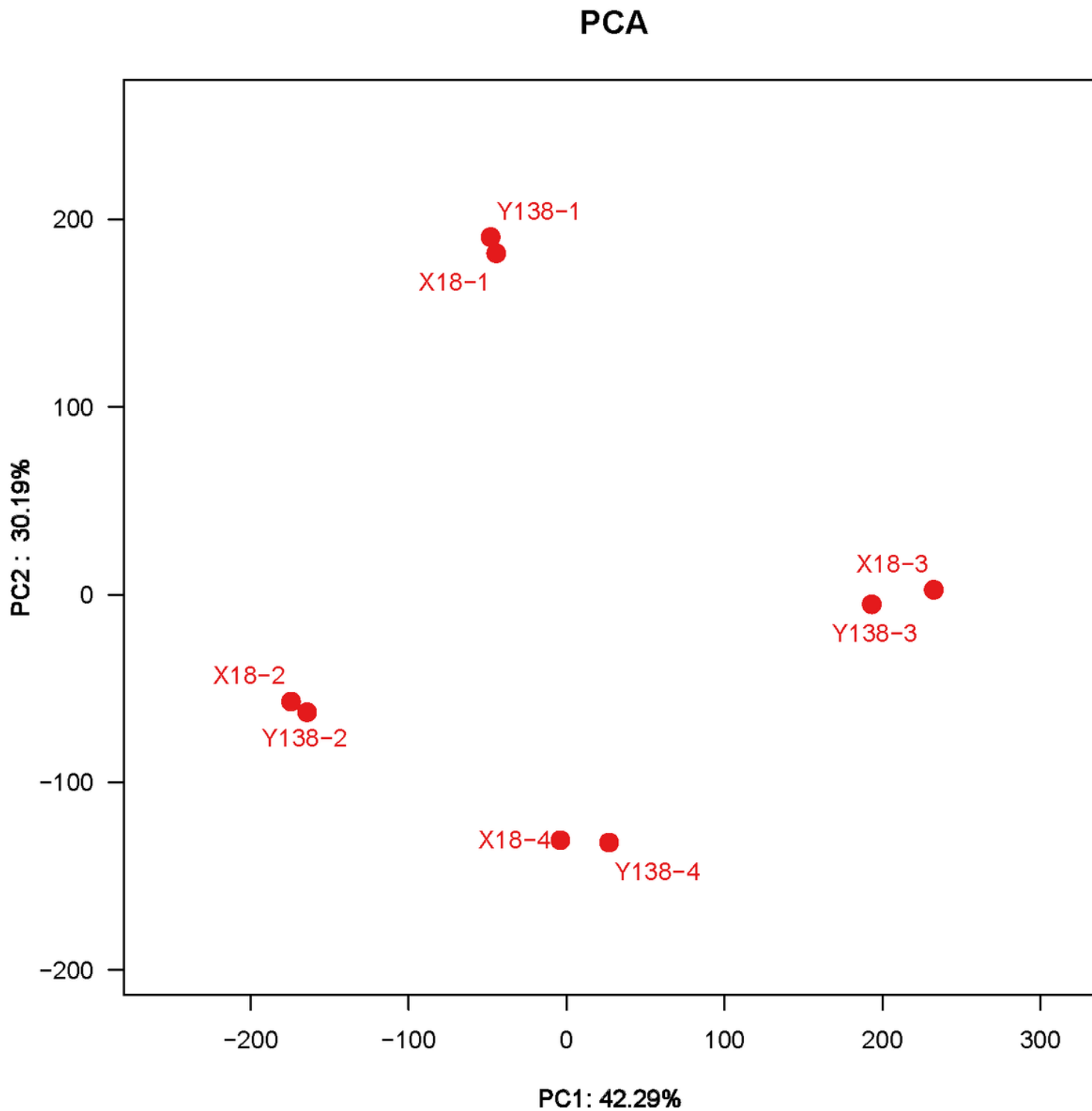
**PCA**

**Figure 6**

Principal component analysis (PCA) of operational taxonomic units. X18: Xushu 18; Y138: Yizi 138; 1 and 2 represent sampling of early planting and early harvest in 2015; 3 and 4 represent sampling of early planting and early harvest in 2016, respectively.
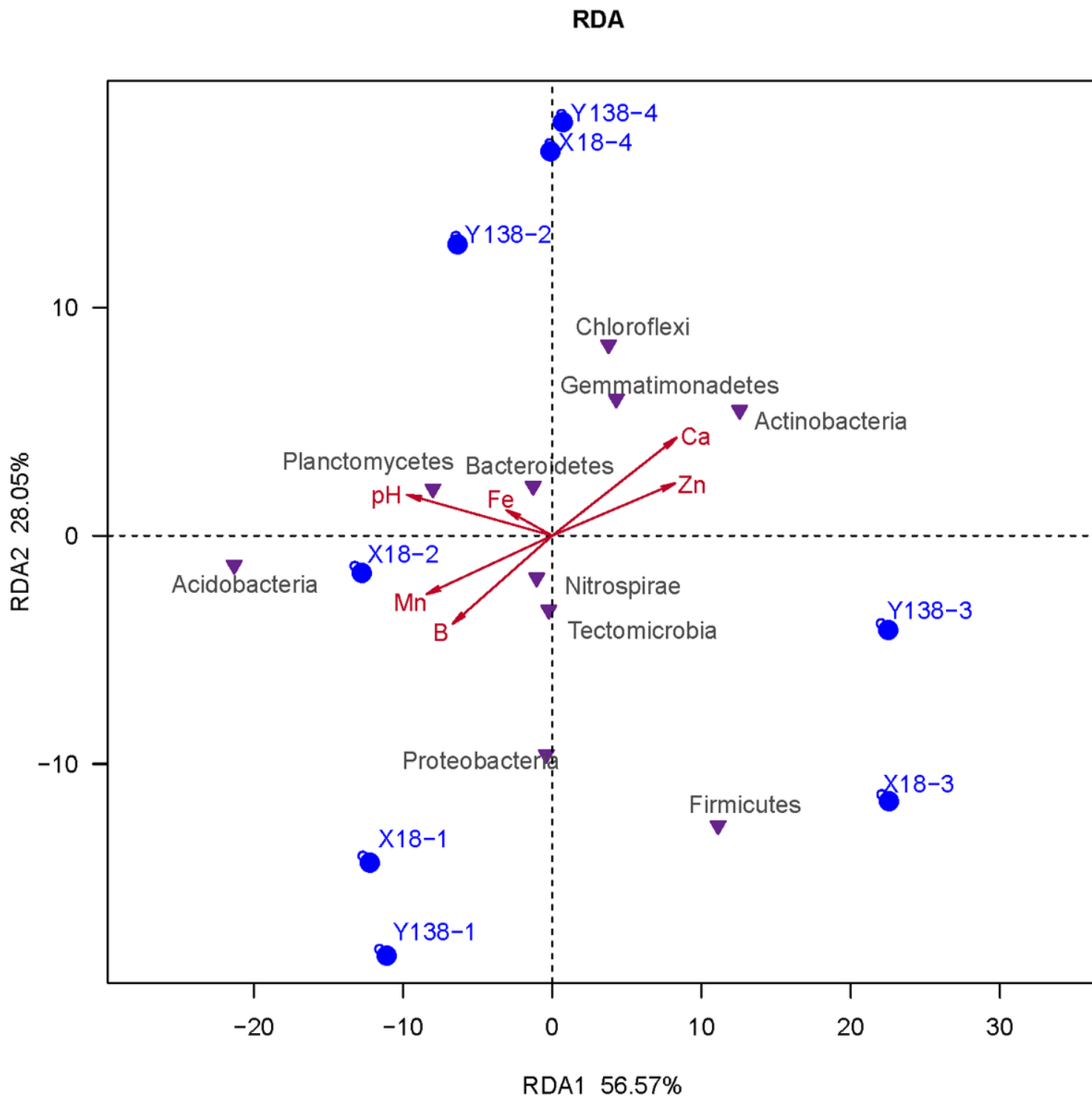
**Figure 7**

Redundancy analysis of the 10 dominant bacterial phyla and soil physicochemical properties. X18:
Xushu 18; Y138: Yizi 138; 1 and 2 represent sampling of early planting and early harvest in 2015; 3 and 4
represent sampling of early planting and early harvest in 2016, respectively; Ca: available calcium; B:
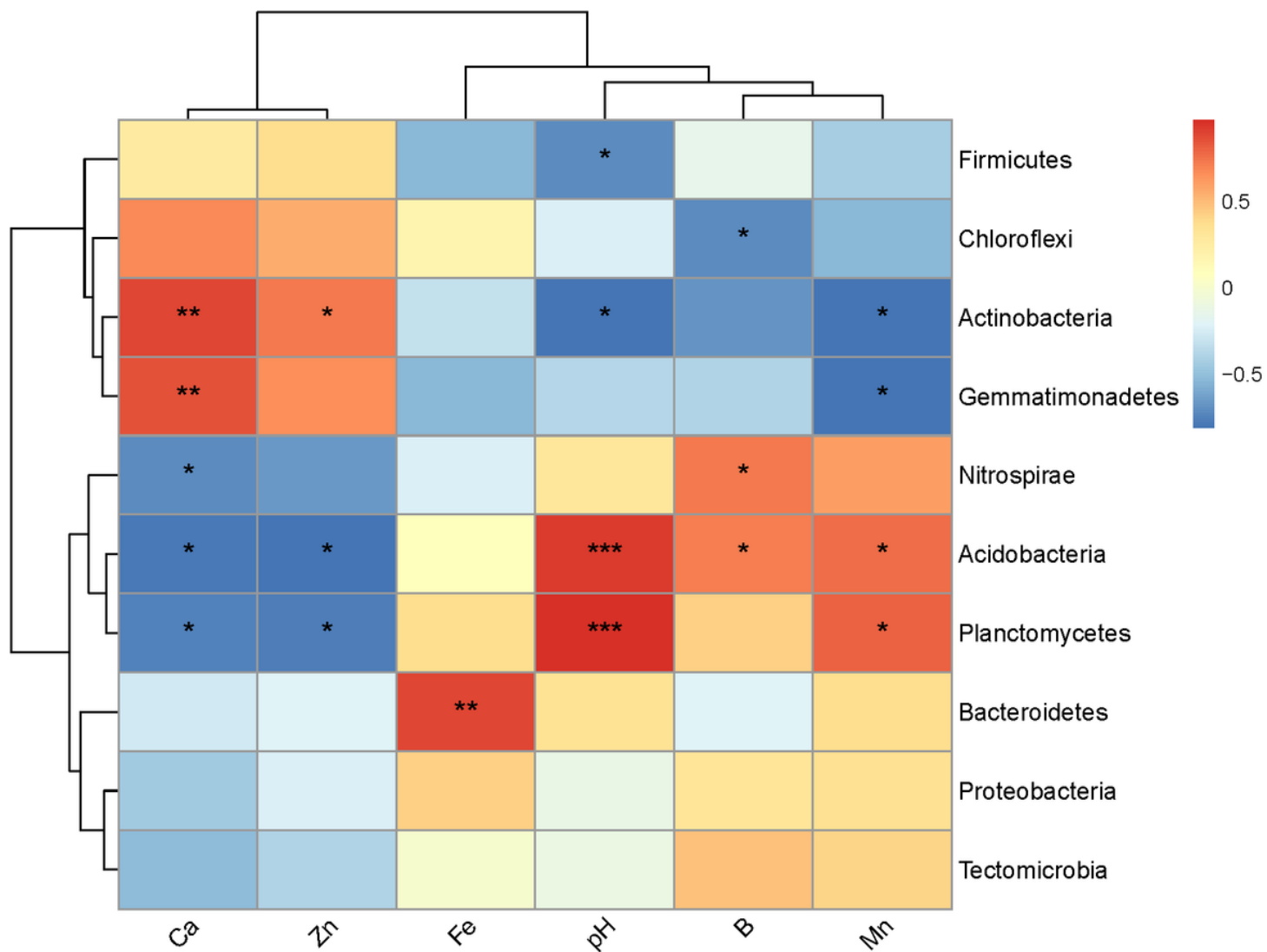available boron; Fe: available iron; Mn: available manganese; Zn: available zinc.

**Figure 8**

Correlations between the 10 dominant bacterial phyla and soil properties. Ca: available calcium; B: available boron; Fe: available iron; Mn: available manganese; Zn: available zinc; *, P<0.05; **, P<0.01; ***, P<0.001.