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

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Research article

Keywords: Bacterial community, continuous cropping, rhizospheric soil, sweet potato

Abstract

Background: Continuous cropping obstacles from sweet potato are widespread, which seriously reduce the yield and quality, cause certain economic losses. Bacteria of Rhizospheric soil are the richest and are associated with obstacles to continuous cropping. However, few studies on how continuous sweet potato cropping affects the rhizospheric soil bacterial community structure. In the study, Illumina Miseq method was used to explore rhizosphere soil bacterial community structure changes with different sweet potato varieties, and the correlation between soil characteristics and this bacterial community after continuous cropping, to provide theoretical guidance for prevention and treatment of sweet potatoes continuous cropping obstacles.

Results: After continuous cropping two years, the results showed that (1) the dominant bacterial phlya in rhizospheric soils from both Xushu18 and Yizi138 were Proteobacteria, Acidobacteria, and Actinobacteria. The most dominant genus was Subgroup 6_norank. The relative abundance of rhizospheric soil bacteria of two sweet potato varieties changed significantly. (2) The richness and diversity indexes of bacteria in Xushu18 rhizospheric soil 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 result indicated that soil pH was correlated significantly with bacterial community. Spearman's rank correlations coefficients analysis demonstrated that pH was positively associated with Planctomycetes and Acidobacteria, but negatively associated with Actinobacteria and Firmicutes.

Conclusions: After continuous cropping, the bacterial community structure and physicochemical properties of sweet potato rhizospheric soil were unbalanced, and the changes from 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 developing new microbial fertilizer for sweet potatoes to alleviate continuous cropping obstacle.

Background

Sweet potato [*Ipomoea batatas* (L.) Lam.] is the main food crop cultivated throughout the world and widely used. It is high-yielding, high efficiency, 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]. Therefore, sweet potato planting area increases annually. In China, due to the limited cultivatable land, sweet potatoes are grown in the same field continuously. However, continuous cropping can lead to decreased yield and quality, severe plant death or even no harvest [5-6]. In recent years, the phenomenon of sweet potato continuous cropping obstacles has become serious, and it has huge adverse impact on the sweet potato industry [7].

Continuous cropping obstacles have a certain relationship with soil enzyme activity, root exudate and soil microbial community [8-9]. Soil microorganisms are the key factors related to changes in soil quality, fertility and productivity [10-12]. The rhizosphere is a soil region near the plant roots, where the interaction between soil microorganisms and the plant root system is very strong [13]. Rhizospheric soil microorganisms are closely related to the absorption and transformation of soil nutrients. Therefore, their community structure is a major factor affecting plant growth, development, and health [14-17]. Many reports indicate that continuous cropping changes the structure of rhizospheric soil microbes [18-21]. These changes have further led to severe continuous cropping obstacles [9]. Therefore, the relationship between them has become one of the research hotspots [7].

Bacteria are abundant in rhizospheric soil and are the important component [22]. Increasing reports have noted that continuous cropping causes bacterial community structure changes in rhizospheric soil. Wu et al. [23] and Wu et al. [24] studied the structure changes of bacterial community in the konjac and vanilla rhizospheric soil after continuous cropping, and revealed continuous cropping changed bacterial communities, resulting in a decrease of beneficial bacteria and an increase of harmful bacteria. Tan et al. [25] studied the diversity and composition changes of rhizospheric soil microbes and endophytes of *Panax notoginseng* after three years of continuous cropping, and found that bacteria decreased over time. Further, the rhizospheric soil bacterial diversity of the healthy *Panax notoginseng* was greater than that of diseased strains. At the same time, CCA analysis found that P, pH, and soil organic matter have the maximum impact on bacterial community. In addition, the results of Na et al. [26] revealed that the bacterial species number and the α-diversity of rhizospheric soil bacterial communities of *Lycium barbarum* L. decreased significantly after continuous cropping, which had great significance to the study of obstacles to continuous cropping.

The detrimental effects on microbial community structures in rhizospheric soil caused by continuous cropping are elucidated 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 used two sweet potato varieties to study the bacterial community structure changes of rhizospheric soil after continuous cropping applying 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 rhizosphere soil bacterial community structure of two sweet potato varieties, and to provide insights into the application of biological control the continuous cropping obstacles of sweet potatoes.

Results

Rhizospheric soil physicochemical properties of sweet potato

After sweet potato continuous cropping two years, available Mn in the X18 and Y138 rhizospheric soil respectively decreased by 32.68% and 31.14% at the beginning of planting, and decreased by 27.35% and

31.10% in pre-harvest period. (**Table 1**). The soil pH of X18 and Y138 respectively decreased by 2.72% and 3.11% at the beginning of planting, the change was not significant in pre-harvest period. Available Ca in X18 and Y138 rhizospheric soil respectively increased by 29.80% and 38.97%, available Zn increased by 56.11% and 43.19% at the beginning of planting, and available Ca increased by 30.75% and 26.47%, respectively; available Zn increased by 29.46% and 30.81% in pre-harvest period. Available Fe of X18 and Y138 respectively decreased by 18.61% and 17.08% in pre-harvest period, the change was not significant at the beginning of planting. Available B in the X18 rhizospheric soil decreased by 20.63% at the beginning of planting, while the change of Y138 was not significant.

Rhizospheric soil bacterial α-diversity

The average coverage of all samples was 96.19% (**Table 2**). Rarefaction curves closed to plateau (**Fig. 1**), indicating that our sequencing depth was good. The reads ranged from 24695 - 37688 for samples, and the OTUs ranged from 3137 - 3734. Chao, Shannon and Simpson indexes were computed based on the bacterial OTU. Unlike Shannon index, the larger the Simpson value, the lower the community diversity. Chao and Shannon values of X18 and Y138 were higher in pre-harvest period than that at the beginning of planting, and they were the opposite of the Simpson index, indicating that two communities had higher species richness and diversity in pre-harvest period. At the same time, X18 had higher Chao and Shannon indices than Y138, which were contrary to the Simpson index. In other words, the bacteria richness and diversity in rhizospheric soil of X18 were higher than those of Y138.

Community composition analysis of rhizospheric soil bacteria

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 X18 and Y138 continuous cropping, Proteobacteria decreased by 17.30% and 8.05% in pre-harvest period, respectively. Acidobacteria showed a decreasing trend and finally increased slightly, while Actinobacteria showed the opposite trend. Firmicutes was higher at the beginning of planting than that in pre-harvest period, while the change of Planctomycetes was opposite. Further, the content of Chloroflexi and Gemmatimonadetes showed an increasing trend. In X18 and Y138 rhizospheric soil, Chloroflexi respectively increased by 81.09% and 96.69%, and Gemmatimonadetes increased by 103.11% and 122.56%, respectively. In addition, compared with X18, Gemmatimonadetes in Y138 rhizospheric soil was higher, especially in 2016.

At the genus level (**Fig. 3**), 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, Subgroup 6_norank in the X18 and Y138 rhizospheric soil showed a decreasing trend, respectively decreased by 54.34% and 52.66%, and then increased slightly in pre-harvest period in 2016. However, *Nitrosomonadaceae*-uncultured and *Anaerolineaceae*-uncultured were present at low levels at the beginning of planting, while increased in pre-harvest period. *Bacillus* and *Lysobacter* showed the opposite trend. Moreover, in every sampling period, *Lysobacter* in rhizospheric soil of X18 was higher than that of Y138. *Bacillus* was the same as *Lysobacter,* except for the beginning of planting in 2015. In addition, in the second year of continuous cropping, *Lysobacter* in X18 and Y138 rhizosphere soil was 1.3 times and 2.4 times of the reduction in the first year.

Venn analysis of rhizospheric soil bacteria

Venn diagrams directly showed the overlapped and unique OTUs of all samples. (**Fig. 4**). After two years of continuous cropping, the OTUs shared by all samples was 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. As continuous cropping year increased, the number of specific OTUs for X18 and Y138 tended to increase. The number of OTUs specific to X18 was more than that of Y138 (except for the beginning of planting in 2016), indicating that continuous cropping led to changes of bacterial communities in X18 and Y138 rhizosphere soil. Further, the differences were largest during the early harvest period of 2016.

Heatmap and clustering analysis and PCA of rhizospheric soil bacteria

The results of heatmap and clustering analysis for 40 phyla of all samples were illustrated in **Fig. 5**. The difference in rhizospheric soil bacterial composition between X18 and Y138 could be seen more clearly. Furthermore, the clustering results showed that the samples grouped into 2 clusters and samples from the same consecutive cropping time 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 continuous cropping time increased, samples from different sampling time were far apart. However, at the same sampling time, X18 and Y138 samples were relatively close to each other. As continuous cropping year increased, 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, the results suggested that (i) continuous cropping led to bacterial community structure changes in X18 and Y138 rhizosphere soil; (ii) rhizospheric soil bacterial community structures of X18 and Y138 were similar in the same sampling period.

Relationship between bacterialphyla and rhizospheric soil physicochemical characteristics of sweet potato

The results of RDA on top ten bacterial phyla and rhizospheric soil environmental factors of X18 and Y138 were showed in **Fig. 7.** RDA1 and RDA2 explained respectively 56.57% and 28.05% of the total variation. These effects of soil properties on bacterial community structure were found in the following order: soil pH > Ca > Mn > Zn > B > Fe. The results showed that soil pH (r^2=0.9737, Pr=0.004), available Ca (r^2=0.8815, Pr=0.011) were significantly correlative with the bacterial community. It indicated that pH was a strong predictor of rhizospheric soil bacterial community for X18 and Y138.

In addition, the results of Spearman's correlation coefficient analysis were as follows (**Fig. 8**): pH was positively correlated with Planctomycetes (R = 0.97) and Acidobacteria (R = 0.93), but had a negative correlation with Actinobacteria (R = −0.79) and Firmicutes (R = −0.72); available Ca was positively related to Actinobacteria (R=0.89) and Gemmatimonadetes (R=0.86), and was inversely correlated with Acidobacteria (R = −0.79), Planctomycetes (R= −0.75) and Nitrospirae (R= −0.72). 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 rhizospheric soil bacterial community structure of sweet potato, Illumina Miseq method was adopted in this study [29-30]. The V4 - V5 highly variable region was selected as sequencing region because a previous study proved that this region was the best sequencing region among nine highly-variable regions [31].

After X18 and Y138 continuous cropping, the most dominant phylum in rhizospheric soil was Proteobacteria, which was in accordance with the published reports [8, 22, 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 came in accordance with Yin et al. [22]. Meanwhile, as continuous cropping time increased, the content of Proteobacteria decreased, which was in accordance with Liu et al. [35]. Acidobacteria showed a tendency to decrease gradually and then increase slightly. Actinobacteria tended to increase gradually and then decrease slightly, which was almost consistent with Yin et al. [22] and our previous study [36], but differed from 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 different continuous cropping years, and plant species, plant genotypes and soil types could also cause different changes in the soil microorganisms [13, 37-39].

This study also found that some microbial changes were affected by sweet potato growth stage. For instance, the content of Firmicutes was high at the beginning of planting and decreased in pre-harvest period. 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 an important impact on soil microbes, but that different microbes vary with season.

As continuous cropping time increased, the content of *Lysobacter* decreased. It is well known that *Lysobacter* is an important biocontrol bacteria with strong bacteriolytic and bacteriostatic effects [41]. For example, *Lysobacterenzymogenes* OH 11 was 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], was found to increase.

After continuous cropping, the bacterial community structure was imbalanced, which was consistent with previous reports [8, 23-24]. These changes further increased the continuous cropping obstacles of **X18 and Y138. In addition, the average yield of X18 and Y138 both decreased, X18 decreased from 12.36 to 10.64** t hm^{-2}**, and Y138 decreased from 9.15 to 2.66** t hm^{-2}.

Consequently, maintaining a balance in the structure of bacterial community is very important for prevention and control continuous cropping obstacles. In practice, we can reasonably apply microbial fertilizers and soil modifier [42-44] to maintain the microecological balance.

The differences of rhizospheric soil bacterial community composition between the two sweet potato varieties after continuous cropping were found. After continuous cropping, 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 bacterial richness and diversity. 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. Moreover, *Lysobacter* and *Bacillus* in the X18 rhizosphere 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.

As sweet potato continuous cropping, rhizospheric soil physicochemical properties were also unbalanced. Moreover, it was found that pH had the greatest impact on bacterial community structure, which was consistent with several published reports [8, 45, 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, Acidobacteria was positively correlated with pH, which was consistent with Yang et al. [34]; Further, Actinobacteria was negatively correlated with pH, which was in accordance with previous studies [24, 47]. 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, soil fungi are important decomposers. However, some fungi are strongly associated with plant diseases [49, 50]. The results of our previous study indicated that fungi diversity and abundance in rhizospheric soil were significantly increased after continuous cropping. Furthermore, the

contents of *Fusarium*, *Verticillium*, and *Colletotrichum,* the pathogens of sweet potato, were increased. These changes affected the balance of fungal community structure [7].

Overall, after continuous cropping, the microbial community structure of sweet potato rhizospheric soil was unbalanced, which might be a crucial aspect that contributed to continuous cropping obstacles. Thus, in practice, we should note that maintaining the microecosystem balance of the rhizosphere soil is very important to relieve the continuous cropping obstacles. In addition, continuous cropping obstacles are related to many factors. It is necessary to combine with many other methods to solve this problem. Therefore, we will carry out further in-depth research on the mechanisms of sweet potatoes continuous cropping obstacles.

Conclusions

Sweet potato continuous cropping led to the rhizospheric soil bacterial community structure and physicochemical properties unbalance, and the changes from 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 developing of specialized microbial fertilizer for sweet potatoes. Therefore, further research is needed to see if *Lysobacter* and *Bacillus* could be used to produce microbial fertilizers to mitigate continuous cropping obstacles of sweet potatoes.

Methods

The study area and materials

Our experiment was conducted at the Dishang test station of Institute of Cereal and Oil Grops, Hebei Academy of Agriculture and Forestry Sciences, China (37°56'24.62" N, 114°42'46.96" E) [7]. There was 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 was Xushu 18 (X18) that was resistant to continuous cropping and the other was Yizi 138 (Y138) that was 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.

Experimental design and sample collection

Sweet potato was planted on May and harvested on October each year, and were continuously planted for 2 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. Details of the plot can be found in our previous report [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 was taken by 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. All

samples were quickly taken to the laboratory with ice box. Each sample was passed through a 2-mm sieve, and then divided the composite soil sample into two portion. One portion was stored at −80 °C for DNA analysis, the other portion was air-dried for soil characteristics analysis.

The soil physicochemical properties analysis

Soil pH was determined by 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 pyrosequencing

The DNA was extracted with an E.Z.N.A. ® Soil DNA Kit following the instructions. The V4 - V5 regions were amplified by PCR with 515F 5'-barcode-GTGCCAGCMGCCGCGG)-3' and 907R 5'-CCGTCAATTCMTTTRAGTTT-3', where barcode was an 8-base sequence unique to each sample. The PCR reactions as well as amplicons extraction and purification were carried out by the method of Yang et al. [34]. Purified PCR products were quantified using Qubit®3.0, and every 24 amplicons whose barcodes differed were mixed equally. The construction of Illumina Pair-End library and the sequencing were according to our previous report [7]. Complete data sets were deposited in the NCBI Sequence Read Archive database under accession number SRP214716.

Processing and sequencing data statistical analysis

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

The Chao, Shannon and Simpson indexes were calculated with Mothur v.1.21.1 [53]. PCA was performed with R-forge (the PCA graphics was generated with Vegan 2.0 package). The shared and unique OTUs were analyzed using Venn diagrams by VennDiagram. RDA was performed to analyze the correlation between environmental factors and bacterial communities. Spearman's rank correlations coefficients and Heatmap figures were performed with Vegan packages in R. The data of the physicochemical properties of rhizospheric soil were analyzed by one-way analysis of variance (ANOVA) ($P < 0.05$) through SPSS v21.0 [34].

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.

Competing interests

The authors declare that they have no competing interests.

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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Abbreviations

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

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Tables

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

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

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

Figures

Figure 1

Rarefaction curves of all samples. 1 and 2 represent the early planting and pre-harvest samples in 2015; 3 and 4 represent the early planting and pre-harvest samples in 2016, respectively. The same as following.
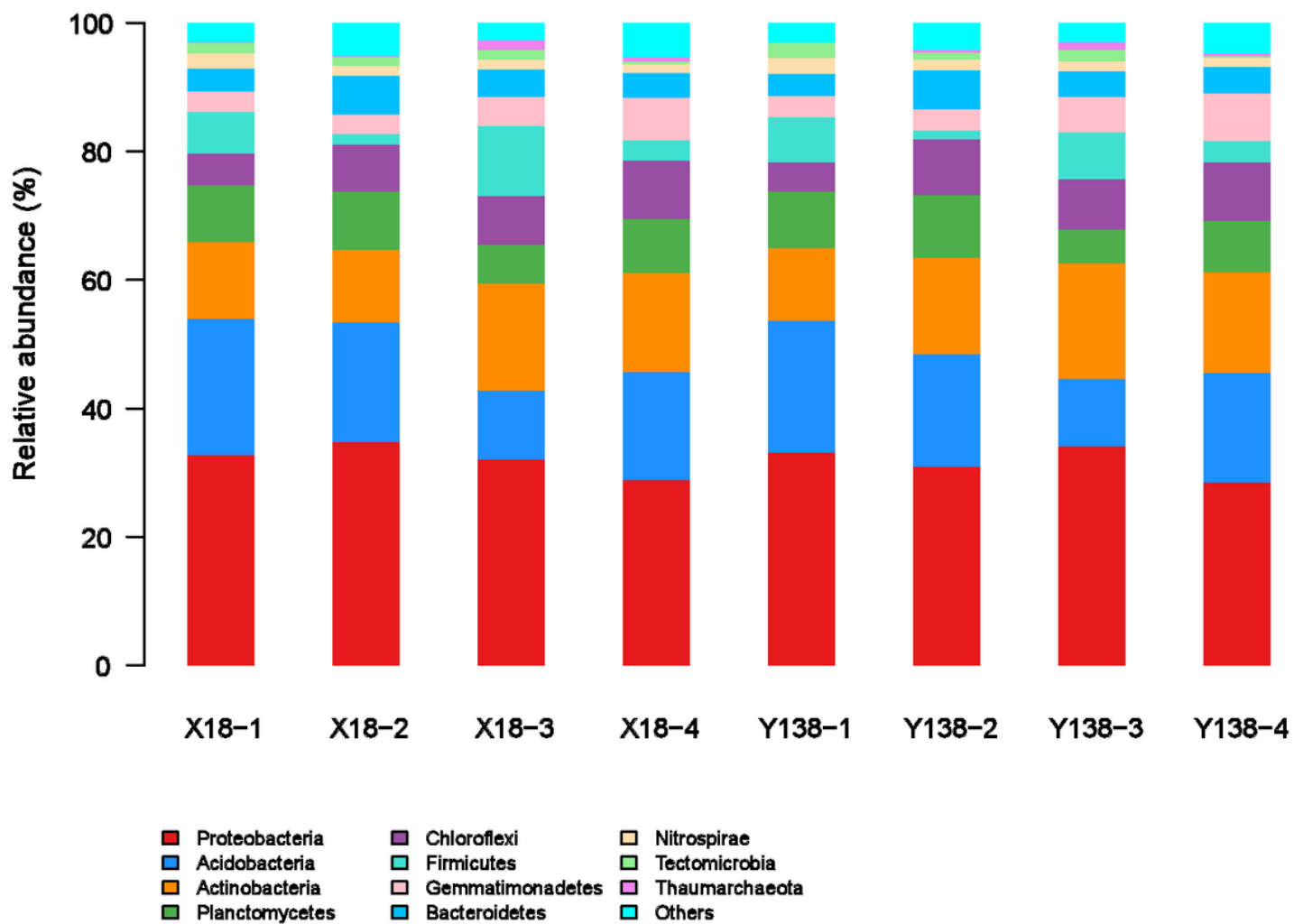
Figure 2

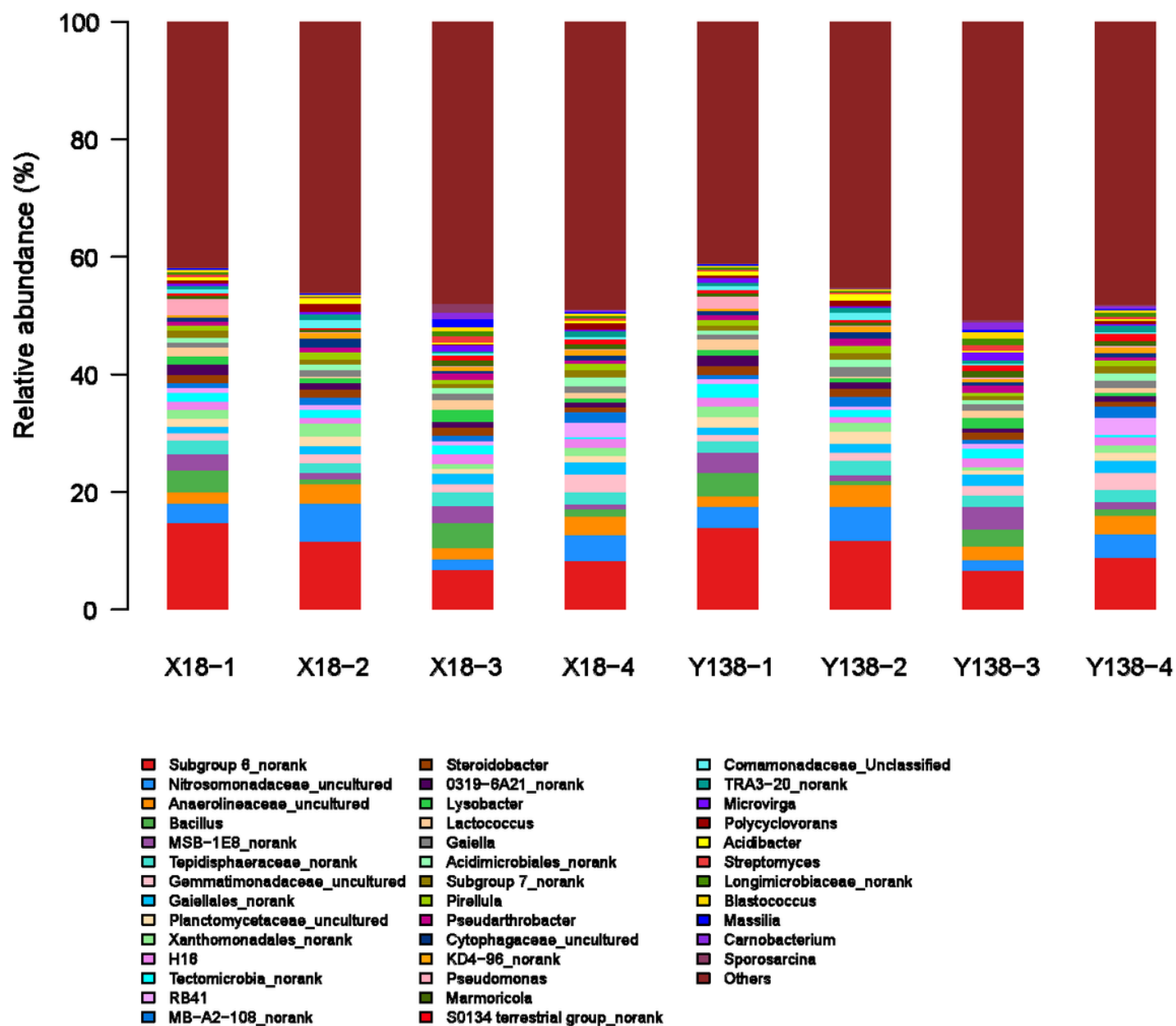Relative abundance of rhizospheric soil bacterial phyla.

Figure 3
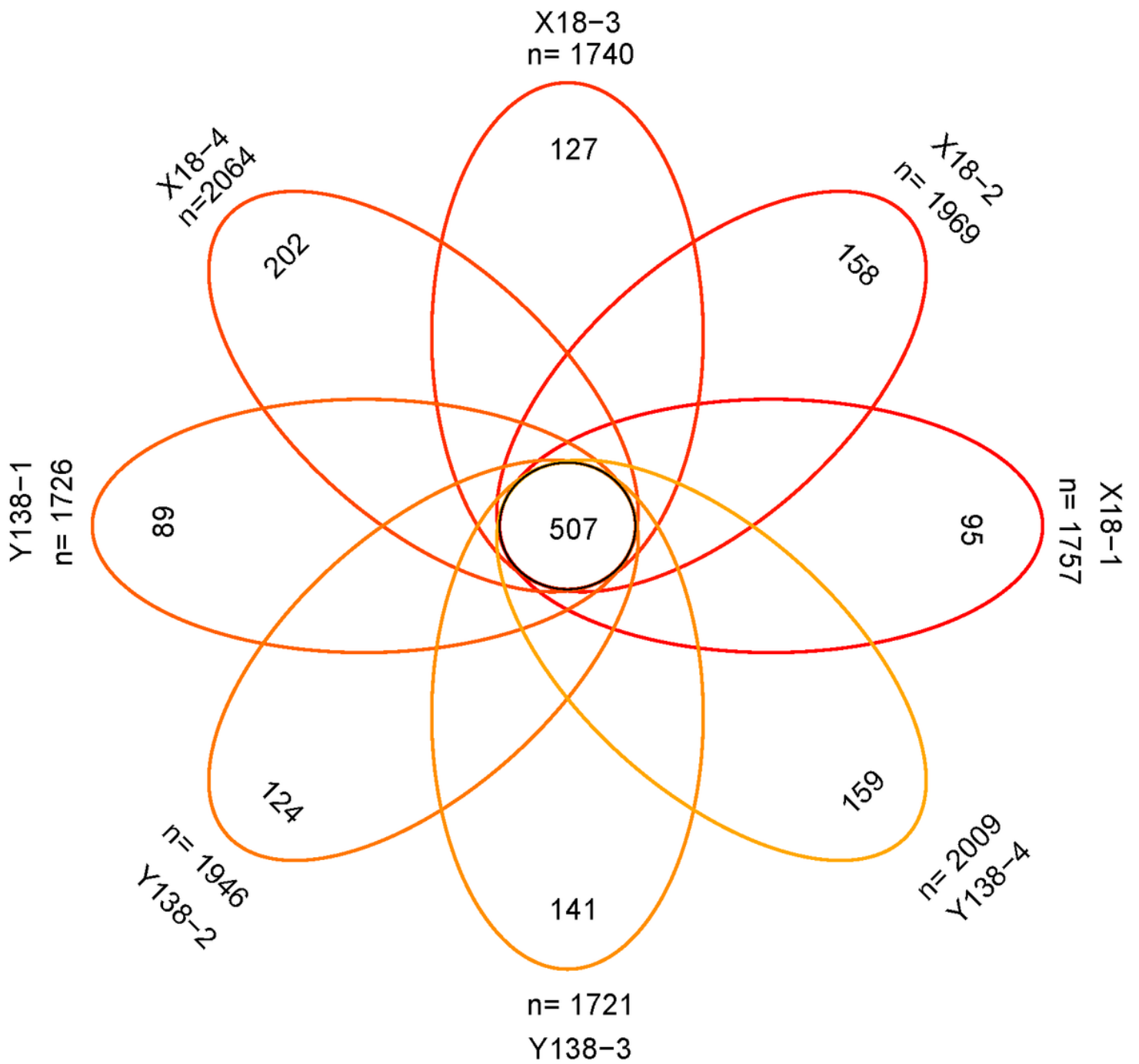
Relative abundance of rhizospheric soil bacterial genera.

Figure 4

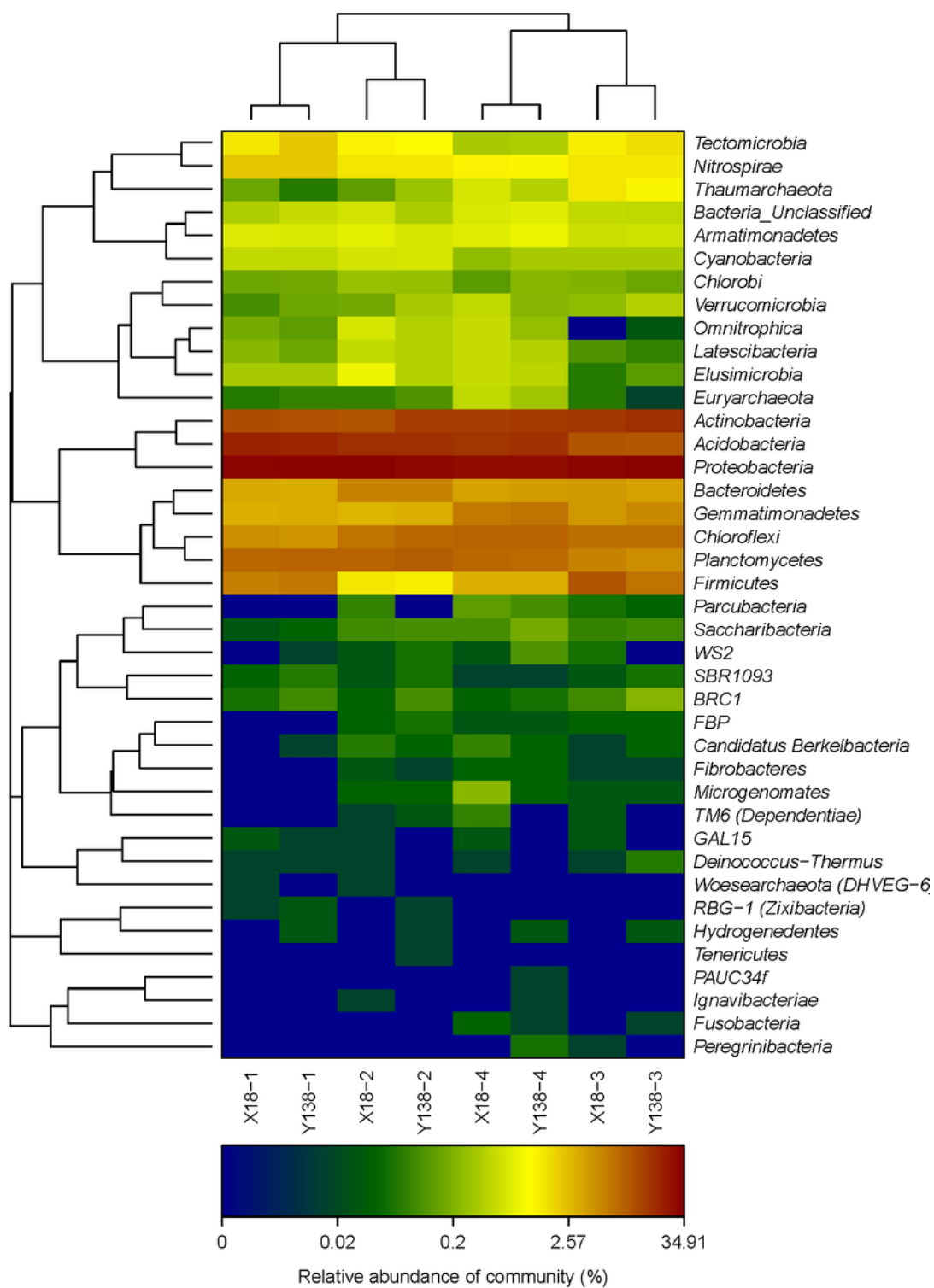Number of common and unique OTUs based on Venn analysis.

Figure 5

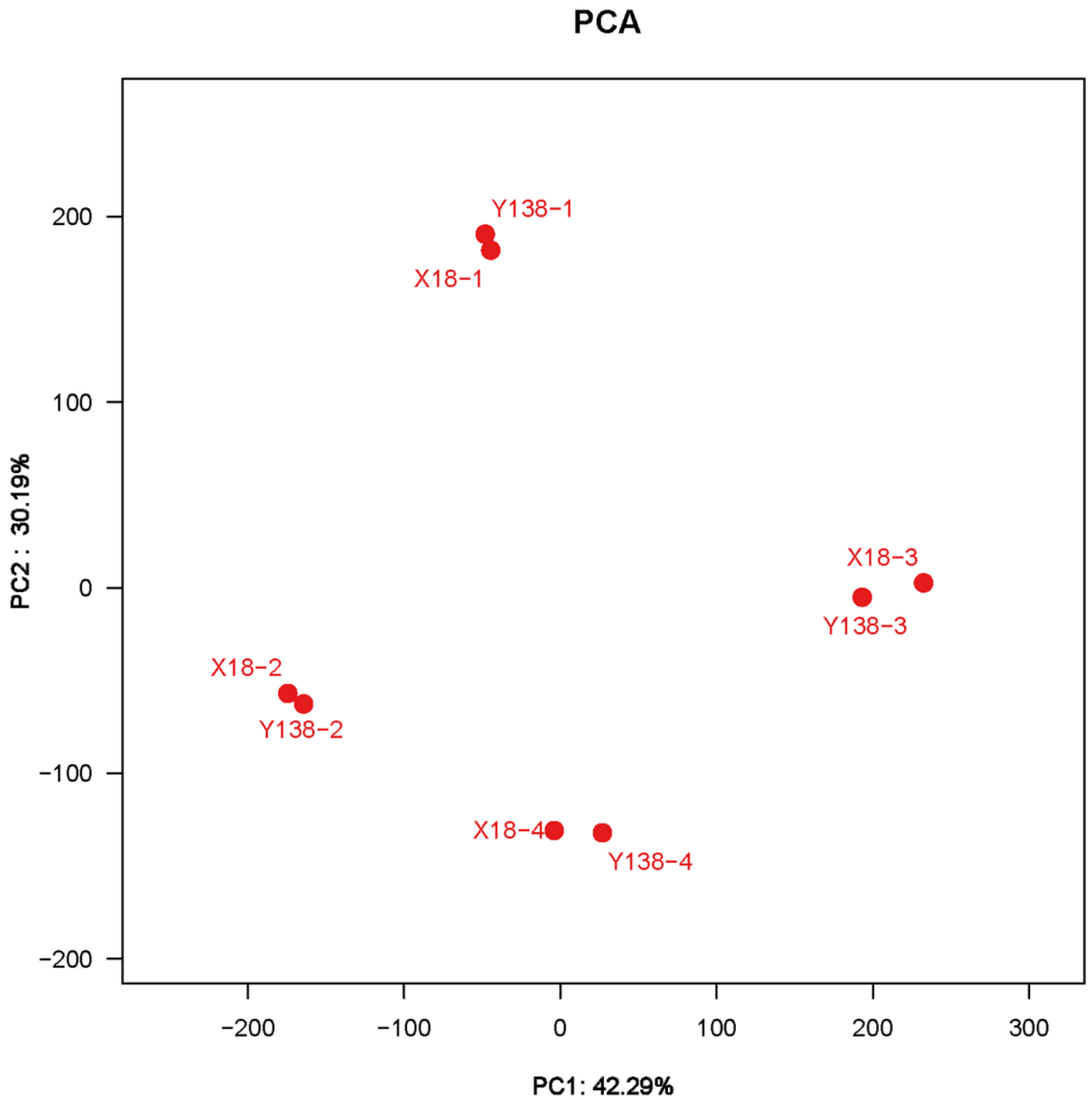Microbial community heatmap and cluster analysis of the bacterial phyla.

PCA

Figure 6

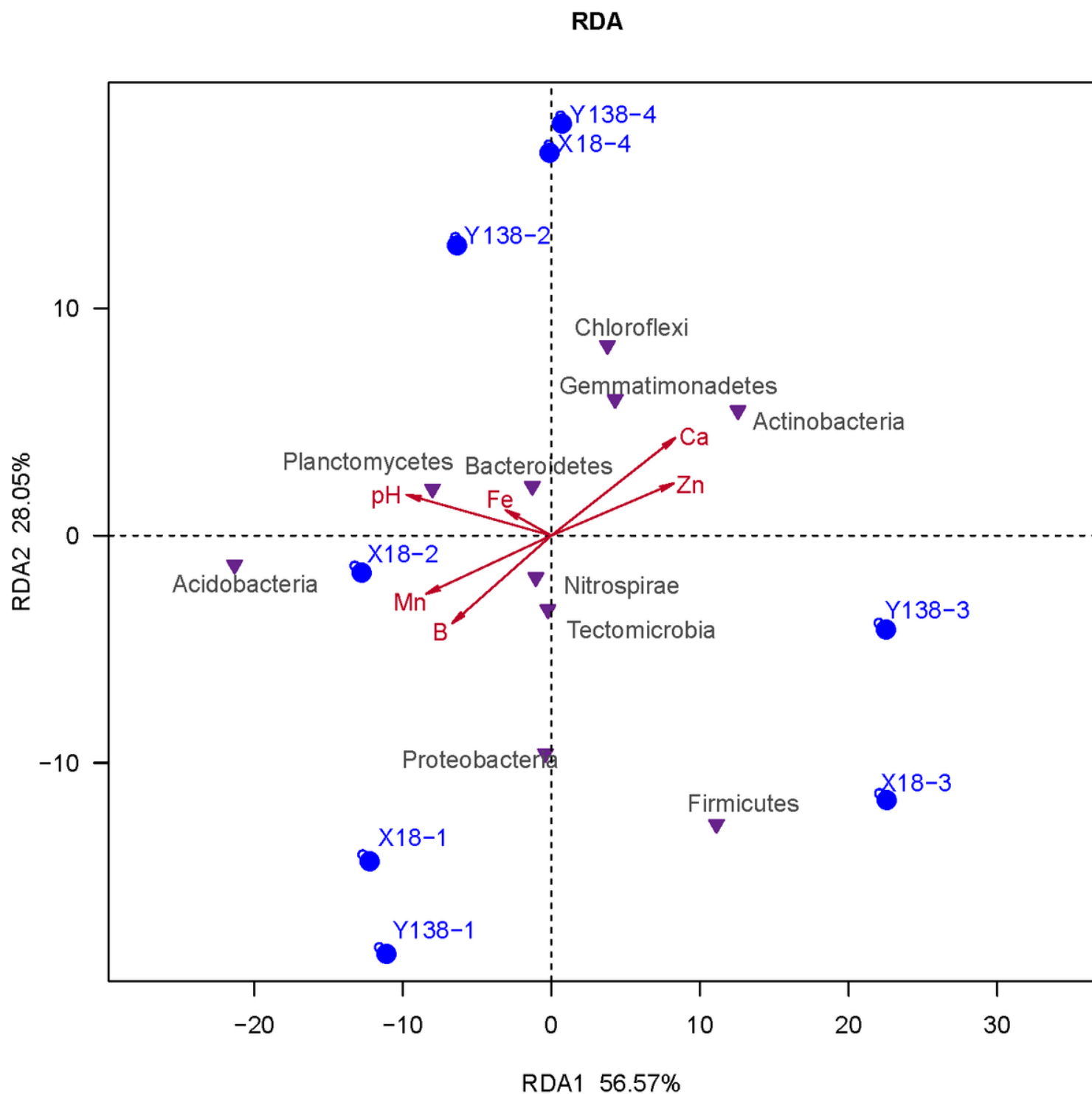Principal component analysis (PCA) of OTUs.

Figure 7

Redundancy analysis of the 10 dominant bacterial phyla and soil physicochemical properties. Ca: available calcium; B: available boron; Fe: available iron; Mn: available manganese; Zn: available zinc. The same as following.
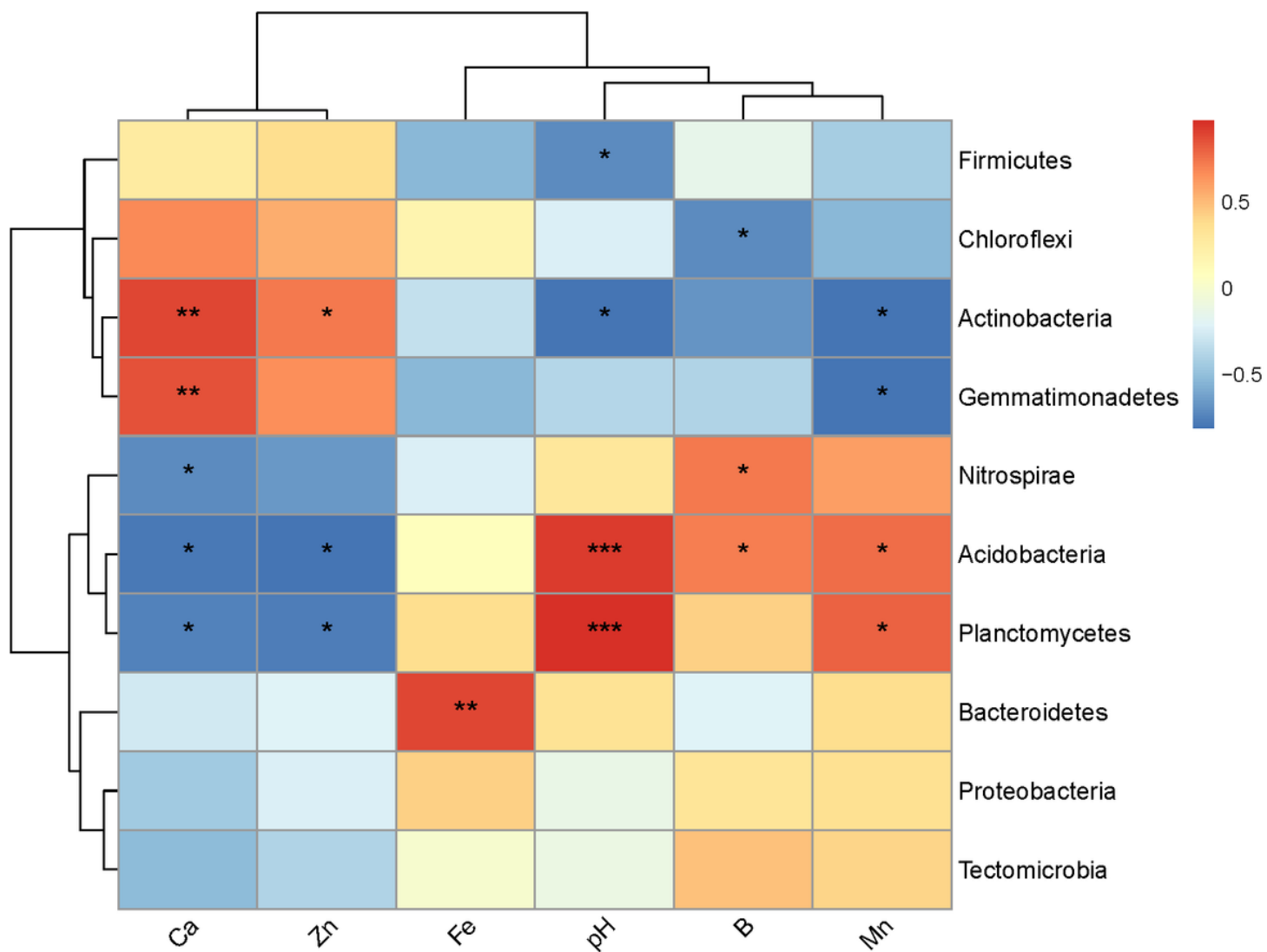
Figure 8

Correlations between the 10 dominant bacterial phyla and soil properties. *, P<0.05; **, P<0.01; ***, P<0.001.