The Rhizosphere Microbial Community of Tobacco and Its Relationship With The Properties of Leaves and Rhizosphere Soils

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Abstract

Background

Rhizosphere microbes possess important effects on plant growth and quality. Here we collected tobacco roots and leaf samples from ten places in Yunnan province to investigate the interaction of the rhizosphere microbes, the soil physicochemical characteristics, and the tobacco leaf properties.

Results

A high-throughput sequencing method was used to sequence the V3–V4 region of 16S rRNA genes, and the operational taxonomic units (OTUs) were clustered using QIIME under 97% identity. A total of 4571 OTUs were obtained from the 30 tobacco root samples, and the top three phyla were Proteobacteria, Acidobacteria, and Actinobacteria, while the top three annotated genera were *Gp6*, *Gemmatimonas,* and *Gp4*. Redundancy analysis (RDA) showed that most of the soil physicochemical properties (10 out of 17) had a significant influence on the rhizosphere microbial community. Both correlation analysis and RDA analysis revealed that quick potassium (K) and Acidobacteria_Gp3 had a significant correlation with the tobacco leaf properties. The variance partitioning analysis showed that rhizosphere microbes had a bigger influence on the tobacco leaf properties.

Conclusions

Our results showed great differences in the rhizosphere microbial diversity of tobacco and complex interaction among the microbial diversity, soil physicochemical characteristics, and tobacco leaf properties.

Background

Nicotiana tabacum is a kind of tobacco which belongs to the class Dicotyledoneac, order Tubiflorae, family Solanaceae, and genus *Nicotiana*. Tobacco originated from America, Australia, and some islands of the South Pacific Ocean and has been planted throughout the world. It was important to the economy of Yunnan province, China [1, 2]. Although it is now well known that smoking is harmful to the health, we cannot deny the medicinal value of tobacco, based on the book, *National Collection of Chinese Herbal Medicine*.

Rhizosphere bacteria play an important role in plant growth, in helping nutrient absorption [3, 4], biotic and abiotic stress resistance [5, 6], and altering the plant physiology [7]. Meanwhile, rhizosphere bacteria are also affected by the root exudates and the soil physicochemical properties [8-10]. Thus, the study of rhizosphere bacteria community composition and the interaction between soil, rhizosphere microbes, and the plant is of great significance. Although the typical culture-dependent bacteria isolation methods could provide some information on rhizosphere bacteria composition and the bacteria strains, the investigation method requires enormous amount of time and labor. Meanwhile, some of the microbes under the state of non-culturable and viable but non-culturable (VBNC) will be ignored [11]. With the development of sequencing technology, next-generation sequencing (NGS) technology has made it possible for in-depth study on the microbial communities and well understanding of the vast diversity and interaction of microbes that have existed in many natural and artificial environmental systems. Culture-independent molecular approaches are frequently used to characterize the compositions and structures of bacterial communities as they are time-efficient and labor-saving [12]. The NGS technology is now widely used in microbial ecology study.

As a bridge to connect the soil bacteria and the bacteria above the soil, plants play important roles in microbial community changes. Diseases of plant leaves, caused by bacteria above the soil, could affect the rhizosphere bacteria community composition by altering the root exudates. In return, the altered rhizosphere bacteria community also could help the plant to resist bacterial pathogen invasion [8]. As an important economical crop, there have been many studies on the control of tobacco diseases and insect pests [13, 14]. Research on the soil bacteria community of tobacco planted under different rotation patterns showed that the soil bacteria diversity decreased after tobacco cultivation, and the proportion of Proteobacteria and Planctomycetes increased, while the relative abundance of Acidobacteria and Verrucomicrobia decreased. The decreased soil microbial diversity might contribute to tobacco bacteria wilt [15]. To control the tobacco bacteria wilt caused by *Ralstonia solanacearum*, antagonistic bacteria were isolated from the soil and plants, and this was shown to decrease *R. solanacearum* in the field experiment [16]. Thus, it is important to study the rhizosphere bacteria community of tobacco as the rhizosphere bacteria play important roles in the plant's growth and stress resistance.

Yunnan province is the largest tobacco production area and produces high-quality tobacco. Here we chose seven cities in Yunnan province to investigate the tobacco rhizosphere bacteria community diversity. In the seven cities, a total of 10 representative places with different soil types, altitudes, tobacco varieties, and intercropping crops were chosen. The main objectives of this study were to investigate (1) the bacteria community composition at different places; (2) the effects of soil physicochemical characteristics on the rhizosphere microbial community; (3) the effects of environmental factors and the rhizosphere microbial community on the quality of tobacco.

Methods

Sampling and sites description

To investigate the rhizosphere bacteria community composition and the relationship with the soil physicochemical characteristics, plant pattern, and tobacco cultivar, a total of ten representative tobacco fields were selected from seven cities in Yunnan province. The sample collection was conducted at the end of July, 2017, when the tobacco plants were in their mature period. The specific location and the environmental factors are shown Table 4. At each sampling site, three points in the tobacco field were selected, and at each point, five healthy plants were chosen for sampling using the five-point sampling method.

The roots of each plant at a depth of about 30 cm were dug out using a shovel. The roots were shaken vigorously, and soil shaken off the roots was collected to test the soil physicochemical characteristics, while the remaining roots were stored in sterile tubes and sent to the lab at 4 °C within 10 hours. Then the roots were placed in 0.85 NaCl solution and shaken for 1 hour. The roots were settled, and the suspension were centrifuged at 9000*g* for 10 min. The pellets from the same sampling point were mixed and stored at -80 °C until the DNA extraction. For each tobacco plant, three healthy leaves, about 50 cm long, were selected and roasted. The first roasted tobacco leaves were used for the leaf physicochemical characteristics measurements.

Soil and leaf physicochemical characteristics measurement

The soils and leaves collected from the same point were also mixed, and then the mixtures were used for physicochemical characteristics measurements. The samples were sent to Yunnan Sanbiao Agriculture and Forestry Technology Co., Ltd. for physicochemical characteristics measurements. A total of 17 physicochemical characteristics (pH, organic matter (OM), hydrolysable nitrogen (SN), available phosphorus (AP), quick potassium (K), available boron (B), exchangeable magnesium (Mg), effective zinc (Zn), hydrolysable chlorine (Cl), effective copper (Cu), effective iron (Fe), effective manganese (Mn), effective sulfur (S), total nitrogen (TN), total phosphorus (TP), total potassium (TK), and exchangeable calcium (Ca)) of the soils were measured according to the national standard method. Meanwhile seven physicochemical characteristics (total sugar (TS), reducing sugar (RS), total nitrogen (TN), nicotine (NT), potassium oxide (PO), hydrolysable chlorine (HC), and starch (ST)) of tobacco leaves were measured according to the national standard method.

DNA extraction, Illumina sequencing and data processing

For DNA extraction, 0.5 g of each rhizosphere soil sample was prepared. The DNA was extracted using a MOBIO PowerSoil DNA Isolation Kit (MOBIO, USA) according to the manufacturer's instructions. The genomic DNA concentration and purity were determined using Epoch (Bioteck, USA) and 1% agarose gel electrophoresis. The final concentration of each DNA sample was adjusted to 1.0 ng/μL using sterile distilled water. Realbio Technology Co. Ltd (Shanghai, China) were entrusted to conduct the library construction and sequencing. The DNA library was constructed using a KAPA HiFi Hotstart ReadyMix PCR kit (KAPA Biosystems, USA) for amplification and AxyPrep DNA Gel Extraction Kit (AXYGEN, USA) for DNA gel extraction. The products were tested and purified using Thermo NanoDrop 2000 (Thermo Fisher Scientific, USA) and using 2% agarose gel electrophoresis. The Illumina sequencing was conducted using primers 341F (5'- CCTACGGGRSGCAGCAG-3') and 806R (5'- GGACTACVVGGGTATCTAATC-3') to amplify the V3–V4 region of 16S rRNA genes. Illumina HiSeq 250 platform was used to generate 2×250 bp paired-ends sequences.

The raw sequences generated from the Illumina HiSeq 250 platform were split into sample libraries based on the barcodes. The reads were trimmed using Btrim [26] with a QC threshold higher than 20 over the 5 bp window size and a minimum length of 100 bp. Forward and reverse reads were joined using Flash [27] with at least 10 bp overlap and fewer than 5% mismatches. UChime [28] was used to remove chimera from those about 425 bp long. Before operational taxonomic unit (OTU) clustering, singletons were first removed from the reads. OTU clustering was determined using Usearch [29] at the 97% similarity level. The taxonomic assignment was conducted through the Ribosomal Database Project (RDP) classifier with an 80% minimal confidence estimate. Subsequent analyses were performed in R using the vegan package [16]. Samples were rarefied at 19,548 reads per sample.

Statistical analyses

The alpha diversity (including the observed species, the Shannon index, the Simpson index, the abundance-based coverage estimator (ACE), good-coverage, and phylogenetic distance (PD)) was calculated using QIIME [30]. The beta diversity of principal component analysis (PCA) was calculated using the vegan package in R software [31, 32]. The correlations between physicochemical characteristics of tobacco leaves with environmental factors and the rhizosphere bacteria community were calculated based on Spearman correlation coefficients in R software. Redundancy analysis (RDA) was performed using the vegan package and plotted using the ggplot2 package in R software [33]. The network analysis was constructed using R software and Gephi software [34]. The analysis of similarities (Anosim) and variance partitioning analysis were conducted using R's vegan package. Functional Annotation of Prokaryotic Taxa (FAPROTAX) was used for function prediction [35].

Results

Physicochemical properties of rhizosphere soils and tobacco leaves

A total of 17 physicochemical parameters at ten different tobacco fields were measured (Table 1). The value of pH ranged from 5.23±0.16 (YX3) to 7.28±0.45 (QJ15) and all samples, except QJ15, were weakly acidic. The heatmap of the rhizosphere soil physicochemical parameters showed that the 17 parameters could be divided into four groups (Fig. 1A). pH, Mg, TN, and OM were in one group and their value (pH) and contents were higher in QJ15. Zn, Cl, and Ca were in a group and their contents were relatively high in QJ15, QJ13, and WS18. Fe, Cu, TP, Mn, and S were grouped together and their contents were high in the YX2 sample. TK, P, AP, and K made the last group and all the four parameters were high in KM1.

For tobacco leaves, seven parameters were measured and the results are shown in Table 2. The heatmap based on the seven parameters revealed three groups of the seven parameters (Fig. 1B). ST, TS, and RS formed a group, while NT and SC formed another group, and TN and PO formed the last one. ST, TS, and RS had higher contents in KM14. NT and SC had relatively higher content in YX2, while TN and PO had higher contents in BS21. For WS18 and YX3, the three repeat samples in each site were not clustered together.

Illumina sequencing and bacterial community structure

The Illumina Hiseq sequencing generated a total of 692,836 clean reads with an average length of 414.6 bp. The reads number of each sample was resampled to 19,548. The OTU numbers of the 30 samples ranged from 1365 (QJ13-3) to 2034 (DL22-1) (Table S1). The rarefaction curve based on the observed species and reads showed that the numbers of observed species for each sample were almost saturated and were sufficient for microbial community analysis (Fig. S1). The mean relative abundances of the top 20 phyla in each sample site are shown in Fig. 2A. Proteobacteria possessed the highest relative abundance in all samples, ranging from 32.50% (DL21-1) to 46.81% (YX3-2) (Table S2). The three dominate phyla were Proteobacteria, Acidobacteria, and Actinobacteria, which accounted almost 80% of the relative abundance. At the genus level, about 40% of the reads could not assigned to a specific genus (Fig. 2B). The top five genera were *Gp6* (6.92%–26.04%), *Gemmatimonas* (2.69%–13.79%), *Gp4* (1.91%–8.11%), *Gp3* (1.95%–7.96%), and *Sphingomonas* (1.54%–7.42%) (Table S3). However, the most abundant genus in each sample was different.

The OTUs in each sample was compared by Venn diagram (Fig. 3). A total of 170 core OTUs were shared by all samples. CX8-1 possessed the most specific OTUs at 15 while YX3-3 possessed no specific OTUs. The specific OTUs of each sample were very few and, for most of them, were lower than 10. The core OTUs and the specific OTUs together with the OTU sequences are listed in Table S4.

The alpha diversity of all samples are listed in Table S5. The highest mean observed species was found in DL22 at 1935 ± 33.79, while the lowest was QJ13 at 1351 ± 19.61 (Fig. S2A). Similarly, DL22 had the highest PD whole tree at 100.04 ± 2.54, while QJ13 had the lowest at 82.44 ± 1.74 (Fig. S2B). All samples had a good coverage higher than 0.97, indicating that the sequence numbers were enough for each sample to perform microbial community analysis.

To illustrate the differences of the microbial communities of the ten sample sites, principal component analysis (PCA) based on the OTUs demonstrated that samples from the same site could have different microbial community profiles (Fig. 4). Samples from Yuxi city YX3 had a more similar microbial community with KM11 than YX2 while KM14 had a more similar microbial community with BS21 than KM11. YX2, QJ15, CX8, and QJ13 had a relative specific microbial community. At the OTU level, PC1 explained 21.32% and PC2, 16.11% of the total variation (Fig. 4).

Relationship among soil properties, rhizosphere microbial communities and tobacco leaf properties

The correlation analysis based on the Spearman method between the physicochemical parameters of tobacco leaves with the soil physicochemical parameters and rhizosphere microbial community at the class level is shown in Fig. 5. Acidobacteria_Gp3 and Flavobacteria had a significant ($P < 0.05$) positive correlation with RS and ST. K showed a significant positive correlation with TS and an extreme significant ($P < 0.01$) positive relationship with ST. Meanwhile, K also possessed a significant negative correlation with PO and an extreme significant negative correlation with NT. Cl also showed an extreme significant negative correlation with ST.

RDA analysis indicated that pH, OM, SN, TN, TP, AP, Cu, Fe, K, and Mg were significantly associated with microbial community diversity (forward selection with a Monte Carlo test, $P < 0.05$). The first two axes explained 25.42% of the microbial community diversity information (Fig. 6A). RDA was also used to assess the relationship between the physicochemical parameters of tobacco leaves with the soil physicochemical parameters and rhizosphere bacterial microbial communities of the top 20 classes (Fig. 6B). Bacteria of Actinobacteria, Deltaproteobacteria, Acidobacteria_Gp4, Acidobacteria_Gp3, Acidobacteria_Gp1, and the soil physicochemical parameters of K, Fe, and Ca were significantly associated with tobacco leaf physicochemical parameters, based on the Monte Carlo test ($P < 0.05$). The first two axes explained 54.95% of the total variance.

The rhizosphere microbial community similarity of different cultivars, different landforms, different soil types, different altitudes, and different rotation crops were analyzed using the ANOSIM method. The results showed that the landform, altitude, and rotation crop had a significant influence in the rhizosphere microbial community, while different cultivars and different soil types showed no significant difference in the rhizosphere microbial community (Table 3).

Variation partitioning of tobacco leaves physicochemical parameters

To investigate the contribution of the rhizosphere microbial community and soil physicochemical characteristics to tobacco leaf property variation, variance partitioning analysis was conducted based on the RDA model and the results are shown using a variation partitioning diagram (Fig. 7). Fifteen classes of bacteria (Bacteroidetes_incertae_sedis, Thermoplasmata, Verrucomicrobiae, Armatimonadetes_gp5, BRC1_genera_incertae_sedis, Candidatus Hydrogenedens, Latescibacteria_genera_incertae_sedis, WPS-1_genera_incertae_sedis, Nitrososphaerales, and six unidentified class) and six soil physicochemical characteristics (pH, SN, Zn, TP, TK, and Ca) were selected as explanatory variables through a forward selection procedure. The rhizosphere microbial community and the soil physicochemical characteristics together could explain 82.68% of the variation in the tobacco leaf properties. The pure effect of the rhizosphere microbial community explained 56.99% of the variation while the pure effect of soil physicochemical characteristics explained 14.77% of the variation. We found that 10.98% of the variation could be explained by the rhizosphere microbial community and soil physicochemical characteristics simultaneously.

Discussion

Due to the important roles the rhizosphere bacteria play in plant growth and stress resistance, investigation of the rhizosphere microbial community has attracted attention. As an important economic plant in Yunnan province, China, it is meaningful to study the rhizosphere microbial community of tobacco. In this study, the rhizosphere microbial community, the soil physicochemical characteristics and tobacco leaf properties were tested and the correlations were investigated. We found that the microbial communities of different cities could be more similar than those from the same city; for instance, YX3 had a more similar microbial community with KM11 than with YX2 (Fig. 4). The rhizosphere microbial community was significantly affected by the soil physicochemical characteristics [9, 10]. The different soil types and rotation crops might contribute to the rhizosphere microbial community diversity of YX2 and YX3.

Like other crops [17-19], the most dominate phylum in all samples was Proteobacteria (Fig. 2A). Nevertheless, the most dominate annotated genus was *Acidobacteria_Gp6* belonging to phylum Acidobacteria and a large proportion of the reads could not be annotated to the genus level (Fig. 2B). Similar bacterial communities were found in a study of tobacco rhizosphere microbial community [20]. In the sugarcane cultivated soils, *Acidobacteria_Gp6* was also the most abundant among the Acidobacteria subgroups and the abundance of *Acidobacteria_Gp6* was decreased with the addition of fertilizer N [21]. However, our results did not suggest a significant correlation of Gp6 and the content of SN and TN (Fig. 5). The investigation of the soil microbial community at the tobacco planting field revealed that *Acidobacteria_Gp6*, *Ktedonobacter*, *Spartobacteria_genera_incertae_sedis*, *Acidobacteria_Gp1,* and *Gemmatimonas* were the top five predominant genera, among which *Acidobacteria_Gp6* and *Gemmatimonas* were also predominant in the rhizosphere microbial community in our study (Fig. 2B).

The high abundance of *Acidobacteria_Gp6* and *Gemmatimonas* in both bulk soil and rhizosphere soil implied an important correlation between them and tobacco. The number of core OTUs shared by all samples was 170, accounting for 3.72% of the total OTUs (4572). The most abundant OTUs belonged to family Sphingomonadaceae, followed by the genus *Arthrobacter*, then genus *Sphingomonas* (Table S4). *Sphingomonas* played an important role in decomposition of toxic chemicals in the soil and maintenance of the soil nitrogen balance [22]. Despite the high abundance and important roles of Sphingomonadaceae, no significant correlation was found between Sphingomonadaceae (belonging to Alphaproteobacteria) with the tobacco leaf properties.

The microbial communities, the environmental factors and the plant physicochemical properties showed strong interactions with each other. K, TN, pH, Mg, Fe, and Cu showed a higher contribution to effect the rhizosphere microbial community than AP, TP, SN, and OM (Fig. 6A). Meanwhile, K showed a significant correlation with the tobacco leaf properties of TS, NT, PO, and ST (Fig. 5). Both RDA analysis and the correlation test showed the influence of the rhizosphere microbial communities and the soil physicochemical characteristics on the tobacco leaf properties, similar to the results already demonstrated in other studies [23-25].

K, Ca, and Fe also showed significant influence on the tobacco leaf properties and showed a higher influence on the sites KM and CX, than on other sites. Actinobacteria, Deltaproteobacteria, Acidobacteria_Gp3, Acidobacteria_Gp1, and Acidobacteria_Gp4 were correlated with the tobacco leaf properties, significantly. Acidobacteria_Gp3, Acidobacteria_Gp1, and Acidobacteria_Gp4 possessed a synergistic effect, and they showed the opposite effect with Actinobacteria and Deltaproteobacteria. Cl showed no significant correlation with the tobacco leaf properties based on the RDA analysis, while showing a significant negative correlation with ST ($R = -0.69$, $P < 0.001$) based on the correlation analysis.

The variance partitioning analysis was used to illuminate the contribution of the rhizosphere microbes and soil physicochemical characteristics made for the tobacco leaf properties. The rhizosphere and soil physicochemical characteristics together could explain 82.68% of the tobacco leaf properties. The rhizosphere microbial communities contributed more than the soil did. As a system, the roots, rhizosphere microbes, and the soil interacted with each other. Most of the soil physicochemical characteristics showed a significant relationship with the rhizosphere microbial communities while only three soil physicochemical characteristics and five microbial taxa at the class level possessed a significant correlation with the tobacco leaf properties, which revealed that the microbes and soils had a stronger correlation than with the tobacco leaves.

Conclusion

In this study, we investigate the rhizosphere microbial communities in ten places from Yunnan province, which is a major tobacco producing area in China. Our results revealed the rhizosphere microbial community diversity in different places. The landform, altitude, and rotation crops presented the most influence in the tobacco rhizosphere microbial community. The top three OTUs belonged to the family Sphingomonadaceae, genus Arthrobacter, and genus Sphingomonas, while the top three genera were *Gp6*, *Gemmatimonas,* and *Gp4*. Most of the soil physicochemical characteristics affected the rhizosphere microbial community and K, Ca, Fe, Actinobacteria, Deltaproteobacteria, Acidobacteria_Gp4, Acidobacteria_Gp3, and Acidobacteria_Gp1 showed a significant influence on the tobacco leaf properties. Meanwhile the microbial community showed a larger influence on the tobacco leaf properties than the soil physicochemical characteristics. However, most results were based on the statistical analysis and specific experiments should be designed and conducted in the future.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets generated in the current study are deposited in the NCBI small read archive (SRA) data set. Accession Link: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA605253. Accession Number: PRJNA605253.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

HXL, QG and XMW conceived the study design. XFY, HYL and ZHZ collected the samples and conducted the experiment. XDL and XL helped to analyze the data and prepared the figures. YYJ, QG and XMW wrote the main manuscript and revised it. All authors reviewed the manuscript.

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Tables

Table 1 The physicochemical parameters of rhizosphere soils for ten tobacco fields (mean ± SD, *n* = 3). Different small letters indicate significant differences between sites for that parameter. The means were compared using pairwise Adonis in R, at the P < 0.05 level.

	BS21	CX8	DL22	KM11	KM14	QJ13	QJ15
pH	6.02±0.27a	6.14±0.05a	6.24±0.09a	5.51±0.04ab	5.62±0.13ab	6.07±0.15a	7.28±0.45c
OM	35.66±1.53b	26.35±0.37ac	36.64±1.06b	29.58±1.71c	53.21±1.56d	25.3±1.15ac	65.2±1.03e
SN	137.84±16.32a	89.35±10.83a	125.62±10.35a	95.73±23.85a	185.79±29.88bd	89.38±8.73a	208.86±31.69cd
AP	18.82±1.46bd	15.24±0.52b	11.41±0.86f	72.28±1.6c	36.94±2.19e	21.02±1.42d	48.87±1.44a
K	106.98±5.48a	325.14±42.22cd	125.46±17.34be	416.34±27.54c	364.27±47.68cd	110.05±2.65ae	178.12±23.67a
B	0.72±0.08ac	0.26±0.04bed	0.62±0.07a	0.83±0.04c	0.4±0.11be	0.61±0.02a	0.07±0.01d
Mg	263.76±48.86f	216.35±22.9bef	254.85±34.33f	80.86±1.56ace	192.46±42.59bcef	117.36±1.54abe	467.46±71.72d
Zn	1.87±0.36e	1.6±0.12b	1.24±0.14be	4.05±0.16ac	4.77±0.54c	2.06±0.11b	3.84±0.54c
Cl	10.81±1e	1.57±0.33a	14.36±1ce	3.6±0.36a	4.77±0.39a	32.83±2.65b	15.75±0.64c
Cu	0.25±0.06g	1.25±0.12b	0.41±0.05g	3.35±0.24c	1.56±0.32bf	1.2±0.09b	2.49±0.36d
Fe	16.25±1.18g	27.14±0.73c	25.35±1.75c	32.14±1.31d	15.14±0.7e	23.23±1.09c	47.77±2.56f
Mn	11.35±0.84e	35.24±0.46c	5.35±0.65f	25.62±1.1d	34.15±2c	34.66±1.19c	21.86±1.16d
S	6.25±0.93e	11.24±0.54cf	7.25±1.07e	8.62±0.45ce	6.24±0.66e	48.69±1.68d	13.55±0.47f
TN	1.25±0.17b	1.35±0.08ab	1.35±0.32ab	1.65±0.09ab	1.36±0.25ab	1.13±0.2b	3.39±0.28c
TP	0.65±0.12bc	1.25±0.51b	0.74±0.08bc	1.24±0.12b	0.52±0.11c	0.85±0.06bc	1.34±0.09b
TK	15.25±0.77cd	13.41±0.86ce	9.53±0.76ae	17.24±0.46d	9.35±0.58ae	8.98±0.87a	11.7±0.52e
Ca	855.64±154.99a	1254.52±139.52ac	951.41±161.11a	895.56±125.01a	1955.41±217.16bcd	2104.06±289.02bd	2281.61±362.97d

OM, organic matter; SN, hydrolysable nitrogen; AP, available phosphorus; K, quick potassium; B, available boron; Mg, exchangeable magnesium; Zn, effective zinc; Cl, hydrolysable chlorine; Cu, effective copper; Fe, effective iron; Mg, effective manganese; S, effective sulfur; TN, total nitrogen; TP, total phosphorus; TK, total potassium; Ca, exchangeable calcium. Units for OM, TN, TP, and TK were g/kg while for the other parameters (except for pH) were mg/kg.

Table 2 The physicochemical parameters of tobacco leaves for ten tobacco fields (mean ± SD, *n* = 3). Different small letters indicate significant differences between sites for that parameter. The means were compared using pairwise Adonis in R, at the P < 0.05 level.

	BS21	CX8	DL22	KM11	KM14	QJ13	QJ15	WS18	YX2
TS	27.14±3.62a	35.76±2.48ab	27.23±7.29a	33.75±0.4ab	44.52±0.71b	29.63±1.34a	29.9±4.24a	38.9±5.26ab	26.3±2.44a
RS	19.17±4.28a	33.61±2.15b	24.29±3.94abc	21.1±2.83a	34.35±0.49c	20.84±3.41a	19.53±4.07a	28.07±5.86abc	21.54±0.53a
TN	2.09±0.08c	1.37±0.07a	1.49±0.19ab	1.6±0.09ab	1.78±0.11b	1.71±0.02ab	1.67±0.11ab	1.62±0.09ab	1.68±0.06ab
NT	2.1±0.08d	1.59±0.09bcd	1.88±0.12d	1.23±0.06bd	1.93±0.09bcd	2.34±0.12cd	1.89±0.03bcd	1.65±0.09d	3.25±0.14a
PO	4.92±0.16e	2.29±0.26ab	2.74±0.13a	1.92±0.3b	1.05±0.05c	2.08±0.12b	1.91±0.04bd	2.47±0.15ab	2.91±0.19a
HC	0.78±0.08d	0.91±0.14b	2.6±0.21e	0.06±0.02c	0.12±0.03c	0.08±0.01c	0.11±0.02c	0.21±0.02c	2.01±0.05a
ST	1.51±0.16f	4.37±0.36c	2.34±0.14a	4.91±0.15c	3.59±0.22e	0.05±0.02d	1.96±0.08af	1.98±0.08af	2.34±0.12a

TS, total sugar; RS, reducing sugar; TN, total nitrogen; NT, nicotine; PO, potassium oxide; HC, hydrolysable chlorine; ST, starch. All units of the parameters were %(w/w).

Table 3 The anosim analysis of rhizosphere microbial community similarity under different environments and plant patterns.

	Cultivar	Landform	Soil type	Altitude	Rotation crop
R*	0.0586	0.2408	0.07871	0.5179	0.4885
P value	0.188	0.02	0.241	0.001	0.001

*R > 0 means that rhizosphere microbial community difference between groups was greater than that within groups.

Table 4 The locations and tobacco field descriptions.

Site names	Cultivar	Landform	Soil type	Longitude and latitude	Altitude (m)	Rotation crop
BS21	Yunyan97	Mountain area	Red soil	99.2186, 25.1875	1610	Winter Rest farmland [a]
CX8	K326	Plain area	Red soil	102.3905, 25.5206	2532	Corn
DL22	Yunyan87	Dam area	Red soil	100.7743, 25.4383	2577	Corn
KM11	Hongda	Mountain area	Sandy loam	103.1416, 25.3480	1900	Pea
KM14	Yunyan87	Mountain area	Red soil	103.1328, 25.4892	2058	Barley
QJ13	Yunyan97	Plain area	Red soil	103.5208, 24.9006	1840	Potato
QJ15	Yunyan97	Mountain area	Red soil	103.8446, 25.2847	1916	Green manure [b]
WS18	K326	Mountain area	Red soil	103.7255, 23.8476	1545	Corn
YX2	K326	Mountain area	Paddy soil	102.7379, 24.2889	1743	Rice
YX3	K326	Mountain area	Red soil	102.1937, 24.6948	1619	Corn

[a] The field was used to plant tobacco only with a winter rest.

[b] The field was used to plant tobacco only with green manure as fertilizer.

Figures

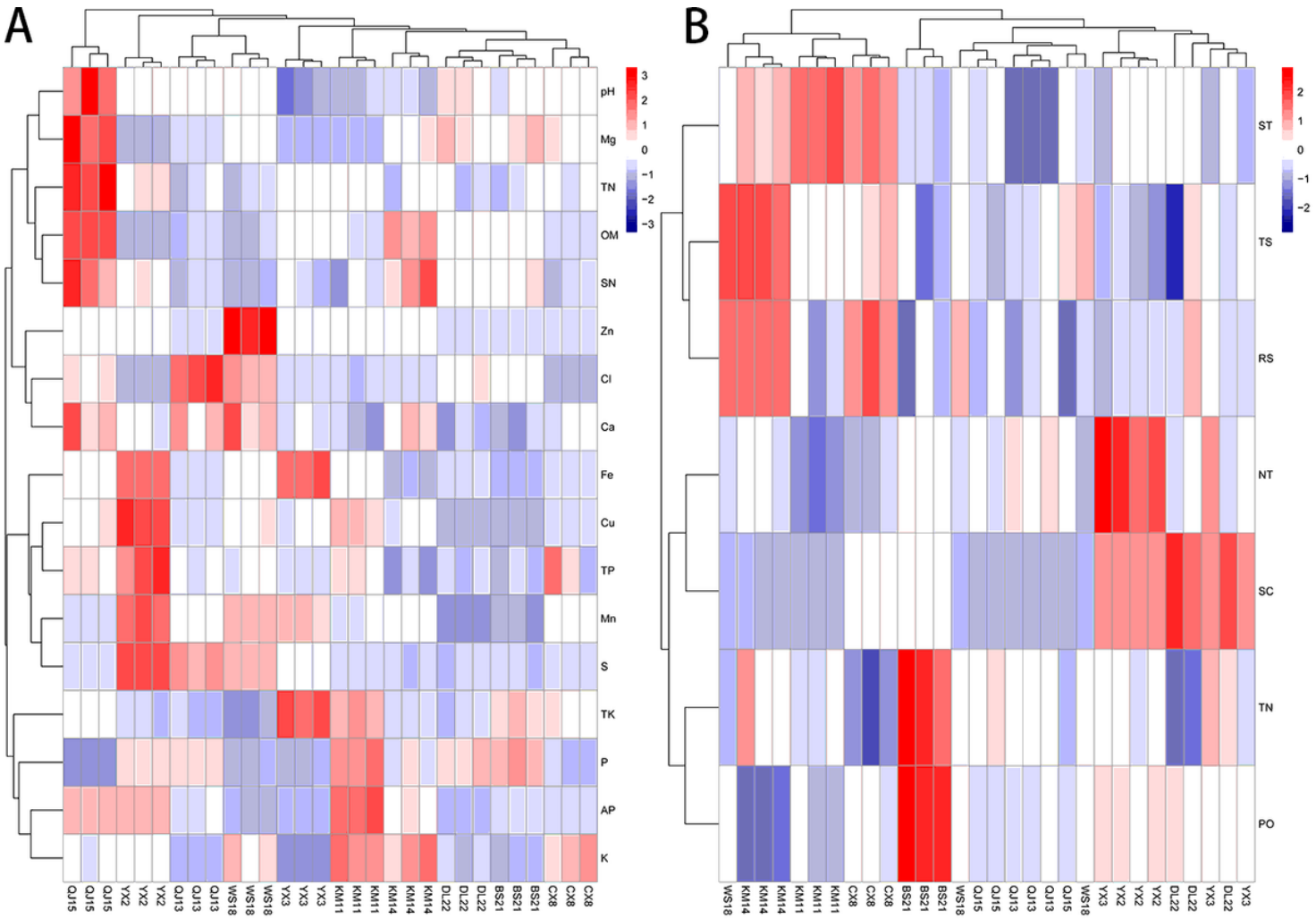

Figure 1

Heatmap between the rhizosphere microbial communities and the soil physicochemical characteristics (A) and the tobacco leaf properties (B). OM, organic matter; SN, hydrolysable nitrogen; AP, available phosphorus; K, quick potassium; B, available boron; Mg, exchangeable magnesium; Zn, effective zinc; Cl, hydrolysable chlorine; Cu, effective copper; Fe, effective iron; Mn, effective manganese; S, effective sulfur; TN, total nitrogen; TP, total phosphorus; TK, total potassium; Ca, exchangeable calcium. TS, total sugar; RS, reducing sugar; TN, total nitrogen; NT, nicotine; PO, potassium oxide; HC, hydrolysable chlorine; ST, starch.

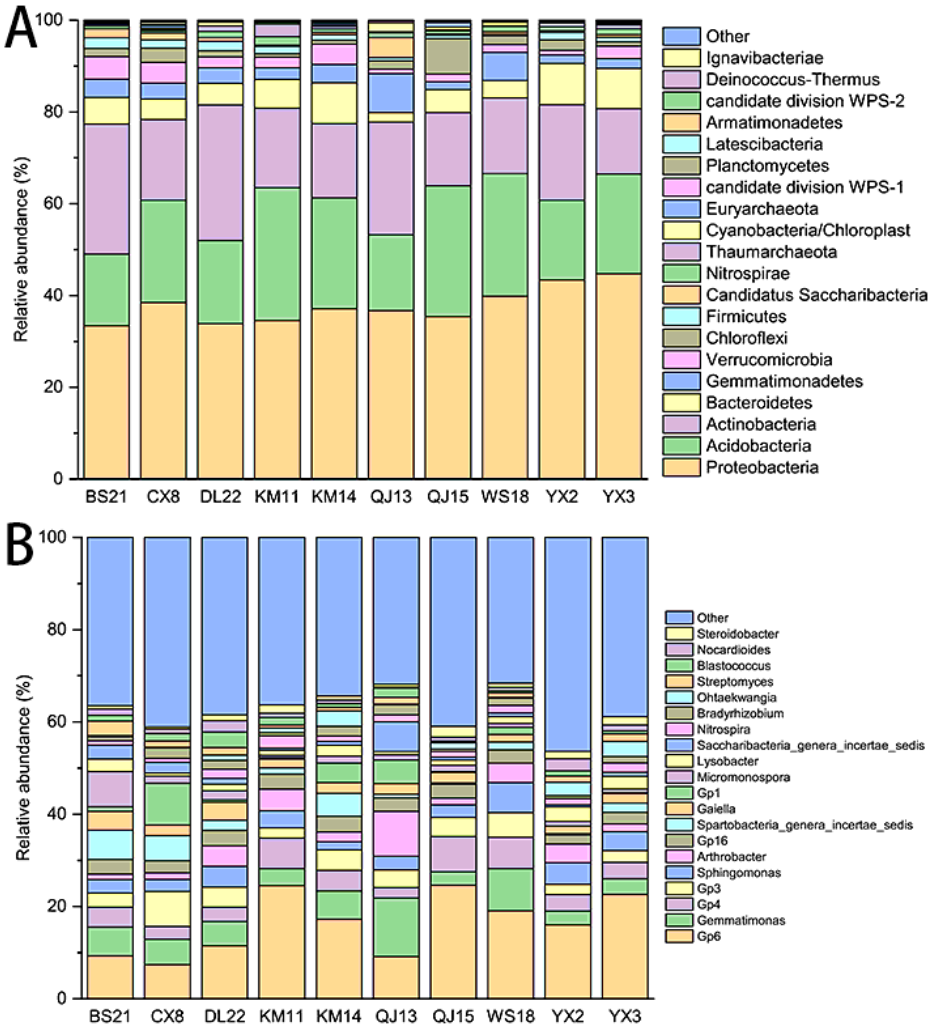

Figure 2

The relative abundance of rhizosphere bacteria community in the ten sample sites at different taxonomy levels. (A): The relative abundance of rhizosphere bacteria at the phylum level. (B): The relative abundance of rhizosphere bacteria at the genus level. The mean relative abundance of the three replicates are shown.

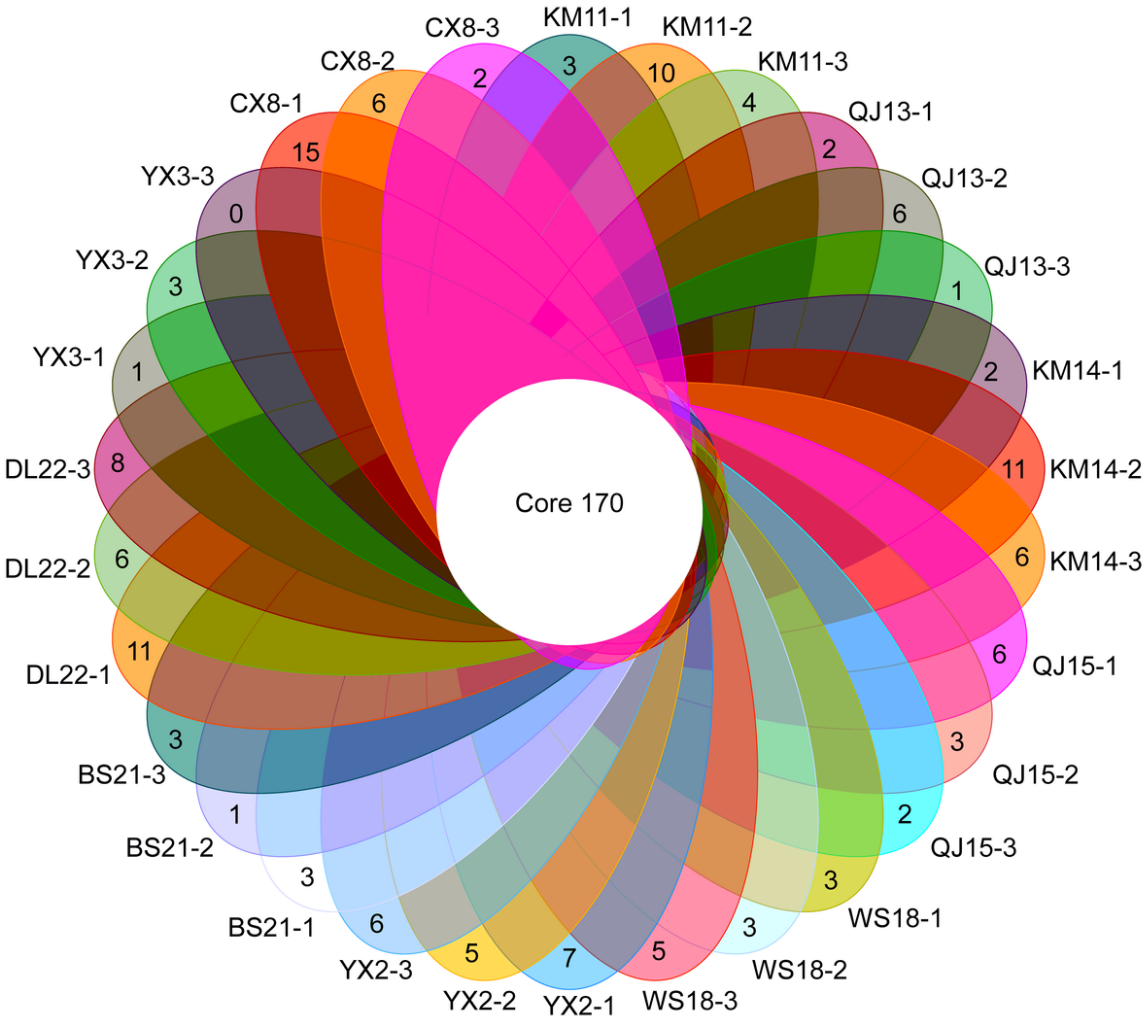

Figure 3

A Venn diagram of OTUs generated from the 30 tobacco root samples. There are 170 core OTUs shared by all samples. The specific OTUs ranged from 0 (YX3-3) to 15 (CX8-1).

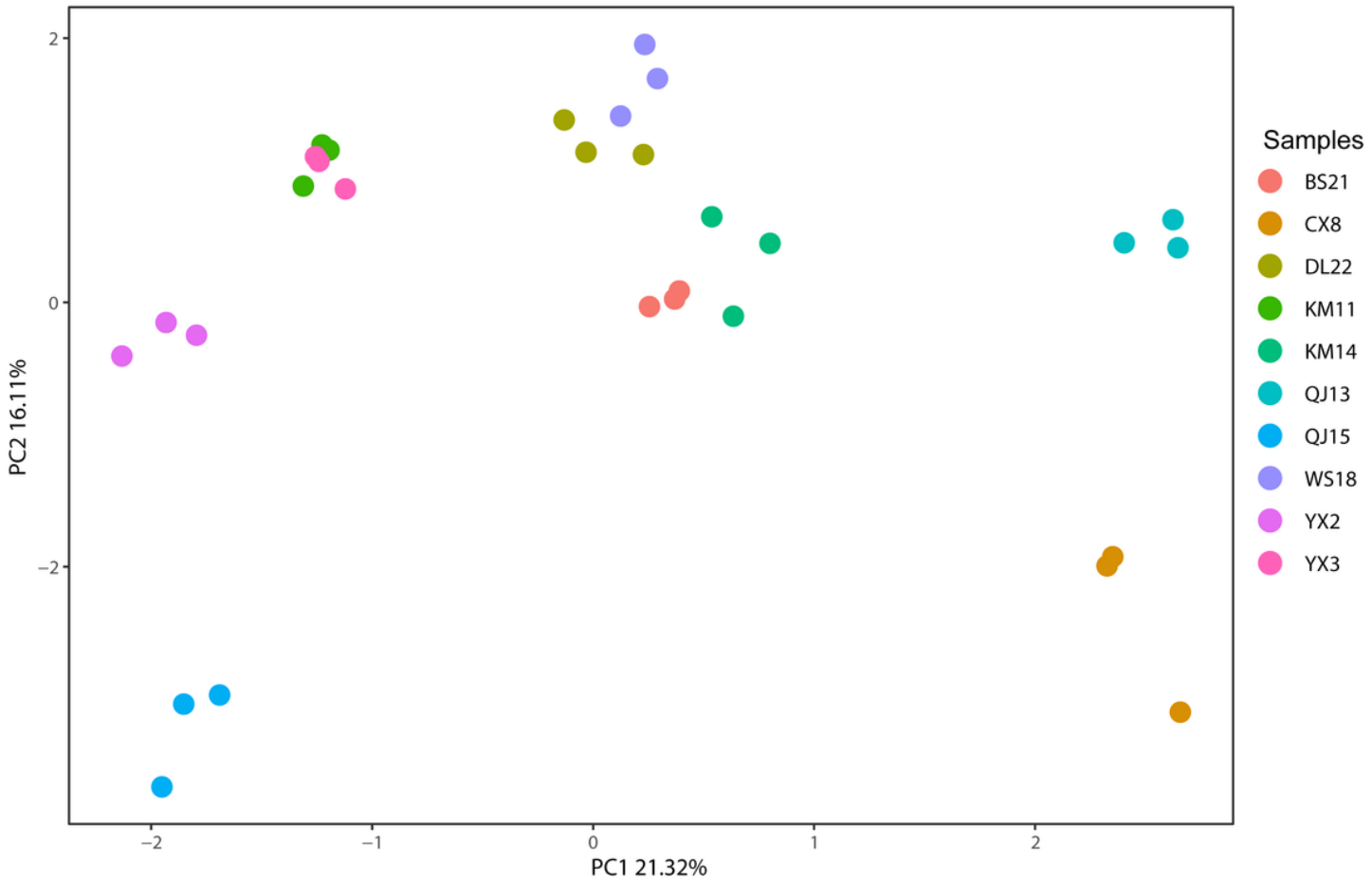

Figure 4

Principal component analysis (PCA) based on the relative abundance of OTUs.

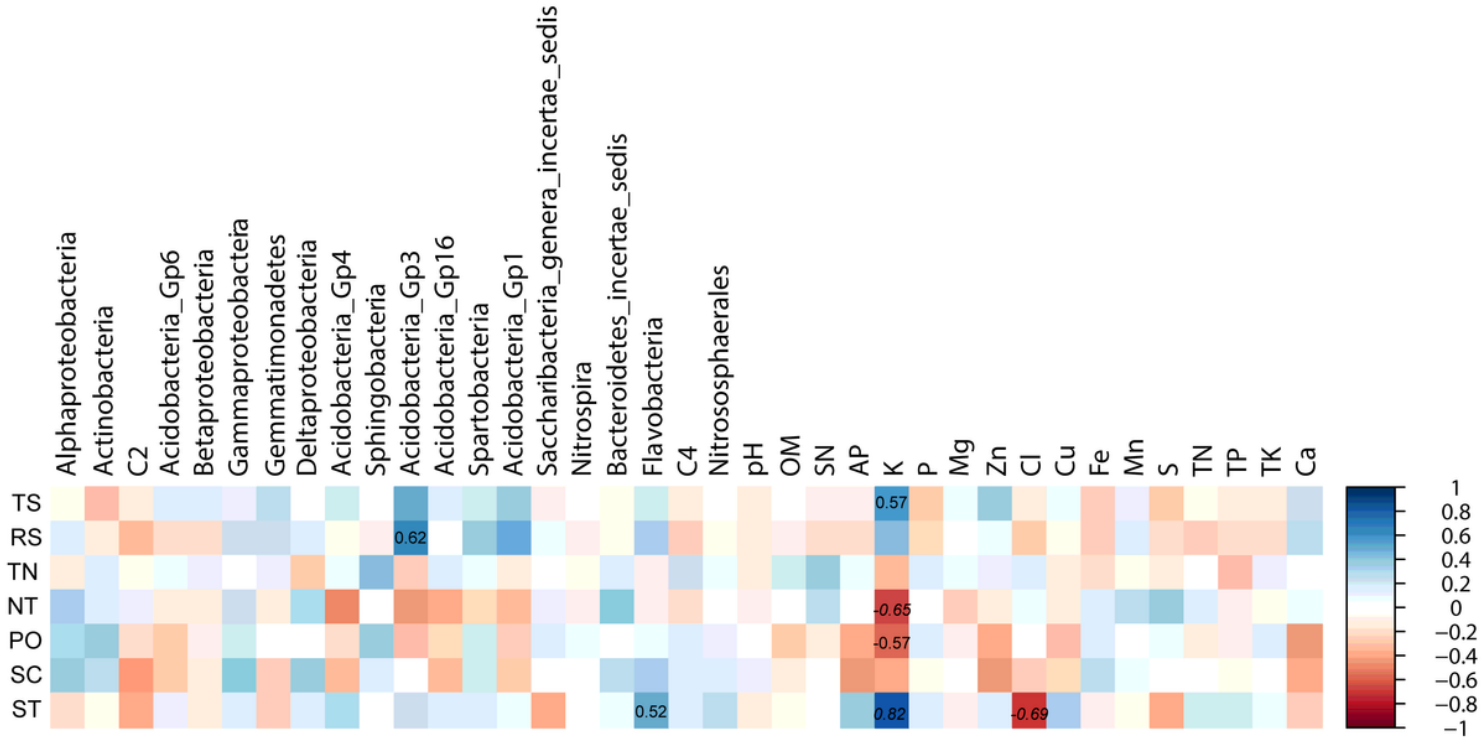

Figure 5

Heatmap of the correlation between the tobacco leaf properties and the rhizosphere bacteria at the class level and the soil physicochemical characteristics based on the Spearman method. Positive and negative values present positive and negative correlations, respectively. The roman and italic numbers represent p-values less than 0.05 and 0.01, respectively.

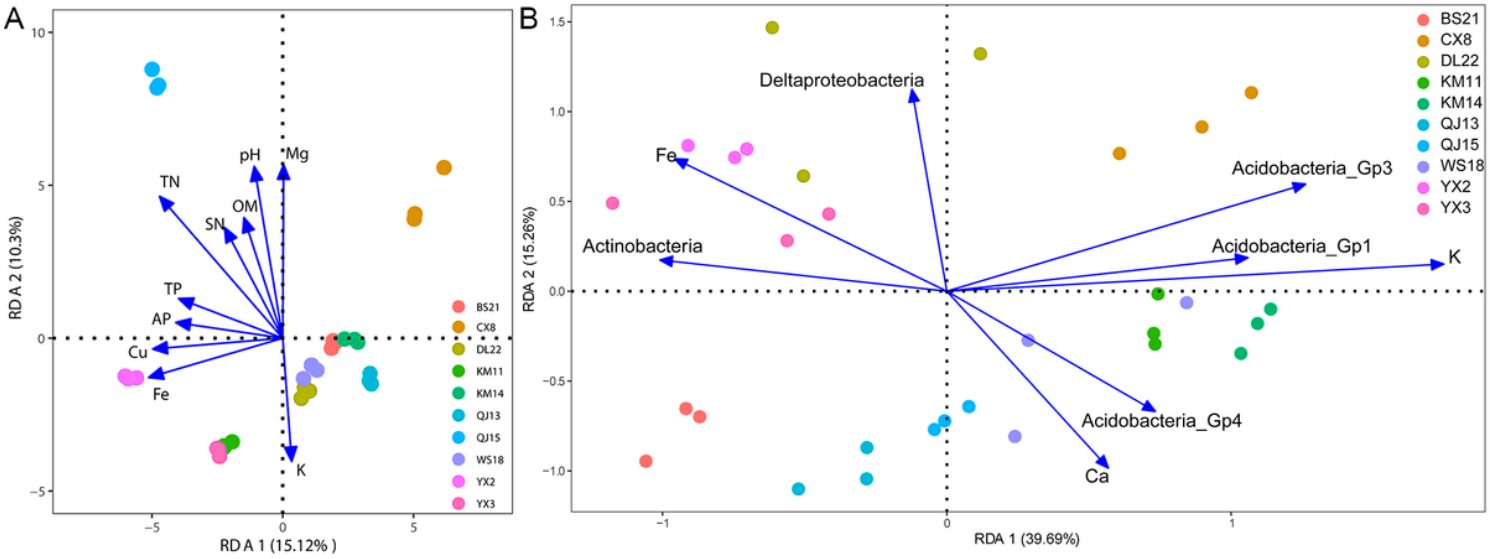

Figure 6

Redundancy analysis (RDA) of the rhizosphere bacteria and soil physicochemical properties (A) and the tobacco leaf properties, the rhizosphere bacteria at class level, and the soil physicochemical properties (B).

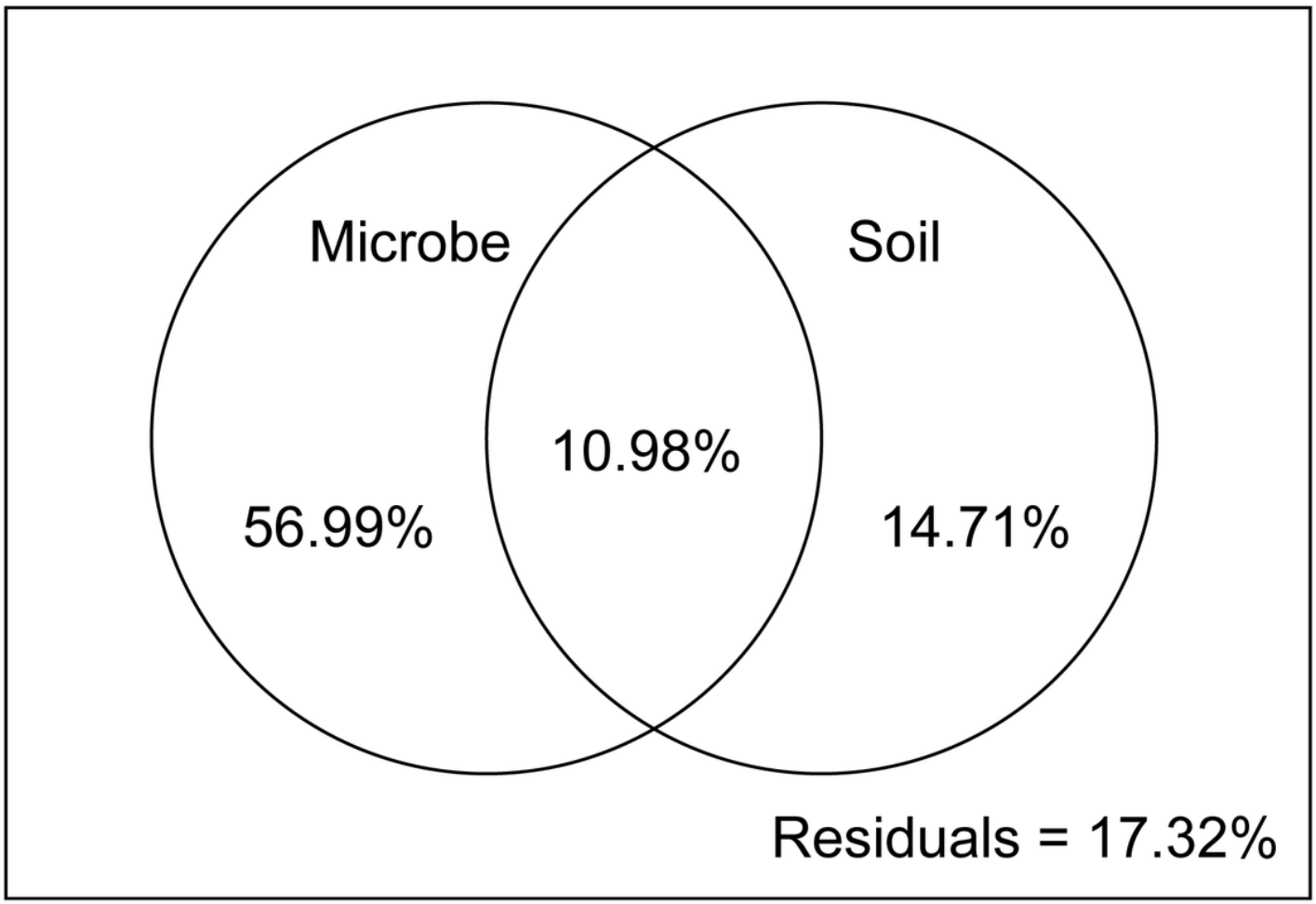

Figure 7

Variation partitioning of the tobacco leaf properties by rhizosphere bacteria community at class level (Microbe) and the soil physicochemical properties (Soil).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementfile.zip