Dynamic changes in the rhizosphere bacterial community in monoculture and intercropping maize and soybean during various crop growth stages

Han Li
Hunan Academy of Agricultural Sciences
Luyun Luo (luoluyun1992@163.com)
Yangtze Normal University https://orcid.org/0000-0003-3513-7229
Bin Tang
Crop Research Institute,Hunan Academy of Agricultural Sciences
Huanle Guo
Crop Research Institute,Hunan Academy of Agricultural Sciences
Zhongyang Cao
Crop Research Institute,Hunan Academy of Agricultural Sciences
Qiang Zeng
Crop Research Institute,Hunan Academy of Agricultural Sciences
Songlin Chen
Crop Research Institute,Hunan Academy of Agricultural Sciences
Zhihui Chen
Crop Research Institute,Hunan Academy of Agricultural Sciences

Original article

Keywords: rhizosphere bacterial community, monoculture and intercropping, ACd

Posted Date: November 25th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-49276/v3

Abstract

Although rhizosphere microorganisms have been studied for a long time, rhizosphere microbial communities based on monoculture and intercropping soybean and maize have rarely been studied. To define the effect of crop monoculture and intercropping on soil physicochemical properties and rhizosphere bacterial communities, field experiments were conducted using maize and soybean cultivars at five different crop growth stages, including monoculture maize, monoculture soybean and maize-soybean intercropping. The rhizosphere bacterial communities were analyzed by using the 16S rRNA Illumina sequencing. The pH and soil organic matter (SOM) were the key factors affecting crop rhizosphere soil bacterial communities. The intercropping soybean-maize increased the available phosphorus (AP) content at five different crop growth stages. And the available potassium (AK) content in the intercropping soybean soil samples was higher than corresponding monoculture soil samples. The content of available cadmium (ACd) in monoculture soybean rhizosphere soil samples decreased and then increased, but the intercropping soybean soil samples indicated an opposite trend. *Proteobacteria, Chloroflexi, Acidobacteria, Actinobacteria* and *Firmicutes* were the dominant phyla in the soybean and maize rhizosphere soil samples. Crops of the same plant species showed little difference in the bacterial community diversity under the two planting modes. The results indicated the intercropping planting pattern altered the absorption of ACd in the maize and soybean soil since the S2 stage and showed a different change in different crop growth stages. And the maize-soybean intercropping system also changed the bacterial community and soil physicochemical properties.

Introduction

Intercropping is often an efficient land use and sustainable agricultural practice and becoming common in the Americas, Asia, Africa, and Europe. It plays an important role in maintaining farmland ecosystem biodiversity and stability (Zhang et al., 2012). Intercropping involves the aboveground and belowground interaction of crops. Intercropping of soybean and maize is a common pattern that can improve maize yield (Zaeem et al., 2019). The maize-soybean relay intercropping system also could effectively increase the grain yield by utilize heat and light resources (Yang et al. 2017; Du et al., 2018). Crops intercropping can increase the N uptake of grain, the nitrogen use efficiency and total N accumulation (Yong et al., 2015; Chen et al., 2017). In recent years, more studies have been focused on the belowground interaction in intercropping systems in altered the soil nutrients, community composition and root exudates (He et al., 2013; Xue et al., 2016). Li et al. (2018) indicated that the maize/peanut intercropping have the advantage of enhancement of soil nutrient, enzymes activity and microbial community composition due to the belowground interactions. He et al. (2013) found that the plant P uptake increased in maize-soybean intercropping due to a shift in the microbial community composition. Changes in soil microbial communities have been observed in mulberry/soybean, maize/chickpea, maize/soybean, and sorghum/peanut intercropping (He et al., 2013; Li et al., 2016; Yang et al.,2016).

The micro-environment in plant roots that interacts closely with soil is called the rhizosphere and includes many components, such as carbohydrates, amino acids and growth substances (Berendsen et

al., 2012). Soil microorganisms can affect and change the supply of soil nutrients by producing various organic acids, hormones, antibiotics, alcohols, vitamins and other products (Manching et al., 2014; Coskun et al. 2017). As an important part of the soil environment, soil microorganisms play an important role in the transformation and increase of soil nutrients and organic matter and thus affect the growth, development and yield and quality of crops (Mendes et al., 2013). The nutrient status of soil can be reflected by the species, number and activity of soil microorganisms (Manching et al., 2014; Yin et al. 2015). Understanding the influencing factors and diversity of the rhizosphere microbial community has great significance for crop growth and contaminated land remediation.

Cd contamination harms the surrounding ecological environment by altering and destroying local ecosystems. Many studies have found that the presence of cadmium will lead to changes in soil properties (Harichová et al., 2012; Hurdebise et al., 2015). In order to find the most crucial stage of cadmium absorption mechanism in intercropping systems, we preliminary explored the dynamic changes of intercropping on physicochemical properties and bacterial community of rhizosphere soil. Therefore, it is imperative to understand the responses of soil microbial assemblages in maize-soybean intercropping system to cadmium contamination. The dynamic changes in the soil physicochemical properties and rhizosphere microbial community of maize-soybean intercropping systems in different growth stages were studied.

Materials And Methods

Site description and sample collection

The experiment was carried out in Zhuzhou City (113°8'3.8"E, 27°43'25.6"N), Hunan Province, China. The field climate was subtropical monsoon humid, with average annual temperature of 17.5-18℃, rainfall of 1400-1500 mm, and sunshine of 1500-1600 h. The field experiment included two monoculture systems, e.g., monoculture maize and monoculture soybean, and maize- soybean intercropping system in a typical cadmium-polluted fallow zone. The compact and high cadmium accumulation maize (Denghai 605) and major soybean cultivars (Xiangchundou 26) of Hunan Province were used as experimental materials. Maize and soybean were sown on April 6, 2019, and harvested on July 30, 2019. The physicochemical properties of the top 20 cm of soil were shown in Table S1.

The planting density for maize was 52500 hm^{-2}, and for soybean was 150000 hm^{-2}. In the monoculture system, the row spacing of maize plants and soybean plants were 60 cm and 33 cm, respectively. And the plant spacing of maize plants and soybean plants were 32 cm and 60 cm, respectively. The distance between maize and soybean rows was 60 cm. Corresponding intercropping systems were planted the same density, while the plant spacing of maize plants and soybean plants were 16 cm and 30 cm, respectively. The ratio of corn to soybean rows was 2:3 in the maize-soybean intercropping system, the plot size was 30 m^2 and each treatment was repeated 4 times. The nitrogen fertilization for maize was divided into two parts, 112.5 kg/hm^2 for base fertilizer and 105.75kg/hm^2 for topdressing. The phosphorus and potassium fertilization were used as base fertilizer at 112.5 kg/hm^2 and 112.5

kg/hm^2 for maize. The nitrogen, phosphorus and potassium fertilization were used as base fertilizer 67.5 kg/hm^2, 67.5 kg/hm^2 and 67.5 kg/hm^2 for soybean. In IMS, the nitrogen topdressing for IM applied with a distance of 40 cm from the maize rows to the soybean rows. The two cultivars were planted in the same experimental field with the same soil properties and climate conditions. There was no irrigation during crop growth.

The rhizosphere soil samples were collected at the V2 (S1), V6 (S2), R4 (S3), R6(S4), R8 (S5) for soybean and V3(S1), V6(S2), VT(S3), R3(S4), R6(S5) for maize (Fehr and Caviness, 1977; Ritchie and Hanway, 1982). The rhizosphere soil was collected by shaking the roots to obtain soil attached to the roots. In each plot, each sample was randomly collected from five points and mixed into one sample.
The rhizosphere soil samples were used for soil microbial DNA extraction, while the rest was air-dried and used for soil property analysis.

Determination of soil physiochemical properties

The soil pH was examined using potentiometry with a pH meter (PB-10, Sartorious, German). The volumetric method was used to measure the content of soil organic matter (SOM) and available nitrogen (AN). UV-Vis spectrophotometry was used to measure the content of available phosphorus (AP). Available potassium (AK) was determined by the inductively coupled plasma-atomic emission spectrometry (ICP-AES).

DNA extraction, PCR amplification and pyrosequencing

In total, 80 rhizosphere soil samples were collected and sequenced. Genomic DNA was extracted using a Fast DNA spin kit for soil (MP Biomedicals LLC, USA) according to the manufacturer's instructions. The extracted DNA was stored at -20 °C before use. The V3-V4 region of the 16S rRNA gene was amplified with primer pairs 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (Kong et al., 2019). Both the forward and reverse primers were tagged with unique barcodes to distinguish different samples. The PCR and sequencing processes were performed by Majorbio Bio-Pharm Technology Co. Ltd., Shanghai, China. Sequencing was performed on an Illumina MiSeq platform using a PE250 kit. The sequencing data were deposited in the NCBI Sequence Read Archive database and the BioProject ID PRJNA662201.

Data analysis and statistical analysis

Data analysis was performed using an open, web-based platform, Galaxy (http://mem.rcees.ac.cn:8080), which comprises an integrated series of bioinformatics tools to aid in intensive bioinformatics research (Kong et al., 2018). Briefly, 12 bp barcode sequences were utilized to sort the different samples. The forward and reverse sequences were then combined with a minimum 30 bp overlap length and a maximum 250 bp maximum overlap length using the FLASH program (Magoc and Salzberg, 2011). Combined sequences with low quality were removed. Subsequently, the reads were clustered into

operational taxonomic units (OTUs) at 97% similarity using UPARSE (Edgar, 2013). The OTU table was resampled with 17,093 sequences to guarantee the same sequencing depth.

The α-diversities (richness, Chao 1, Shannon index, and inverse Simpson index) were calculated, and the relative abundances of phyla and genera were examined in our study. Weighted principal coordinate analysis (PCoA) based on weighted UniFrac matrix and dissimilarity tests (nonparametric permutational multivariate (PERMANOVA) based on Bray Curtis) were performed to investigate differences in microbial community structure (Anderson, 2010; Caporaso et al., 2010). The Mantel test was used to evaluate the correlation of physicochemical properties and the microbial community structure. Significance between groups was determined by one-way analysis of variance with SPSS 22. The student t-test analysis was performed to evaluate the difference of physicochemical properties between the intercropping and corresponding monoculture maize/soybean samples during various crop growth stages by using the Excel 2017.

Results

Soil physicochemical properties at different growth stages

The physicochemical properties of all soil samples collected at 20, 40, 60, 80, and 100 days after the crops were planted at five different stages were measured in succession (Fig. 1). The soil pH of the rhizosphere soil samples from the monoculture soybean all decreased, while that of the maize samples increased; intercropping soybean and maize rhizosphere soil samples all decreased and then increased and showed the lowest value at the S3 stage (Fig. 1). The SOM contents in the monoculture and intercropping soybean rhizosphere soil samples all decreased and then increased, and the SOM content of the intercropping maize gradually increased (Fig. 1). The AP, AN, and AK contents in the intercropping soybean rhizosphere soil samples all decreased from the S1 to S3 stage and then rebounded to normal levels at the S3 stage. The AP and AK contents of the monoculture soybean showed no significant changes during the stages, and the AN content all increased and then decreased; the AN and AK contents of the monoculture maize all decreased and then increased. The AP of the intercropping soybean increased and then rebounded to the normal level at the S3 stage (Fig. 1). The content of ACd in monoculture soybean rhizosphere soil samples all decreased and then increased, but the intercropping soybean soil samples showed the opposite trend. The dynamic trends of the physicochemical properties of the maize rhizosphere soil were opposite to those of the soybean rhizosphere soil (Fig. 1). And all the physicochemical properties were compared in monoculture and intercropping maize and soybean at every sampling time (Table 1). The content of AP in the intercropping soybean/maize soil samples was higher than the corresponding monoculture soil samples at five different growth stages. The content of AK in the intercropping soybean was significantly higher than the corresponding monoculture soil samples at five different growth stages. But content of AK in the intercropping maize was lower than the monoculture soil from S2 to S4 stages. The content of ACd in the intercropping soybean was significantly higher than the monoculture soil from S2 to S4 stages.

Bacterial community diversity and composition at different growth stages

After a series of steps, a total of 3,881,770 high-quality sequences were obtained from Illumina MiSeq sequencing of 80 rhizosphere soil samples. The observed richness (OTU numbers) and Shannon and inverse Simpson indices of monoculture and intercropping soybean rhizosphere soil samples all decreased and then increased, while monoculture and intercropping maize rhizosphere soil samples showed no significant changes at their different stages (Fig. 2). The Chao1 estimated richness of the monoculture and intercropping maize and soybean rhizosphere soil samples gradually increased at the five stages (Fig. 2). In addition, Student's t-test results between the two groups showed significant differences in the α diversity indices between monoculture maize and soybean at the S2, S3 and S4 stages as well as intercropping maize and soybean at the S1, S2 and S4 stages (Table S2). There were also significant differences in the α diversity indices between monoculture and intercropping soybean at the S4 stage and monoculture and intercropping maize at the S2 stage (Table S2).

The principal coordinate analysis (PCoA) (Fig. 3) and PERMANOVA results (Table S3) showed that the monoculture soybean rhizosphere bacterial community structure was significantly different from S1 to S4, while that of the intercropping soybean was significantly different at the S1, S2, S3(S4) and S5 stages. The monoculture and intercropping maize rhizosphere bacterial community structure was significantly different among all five stages ($P < 0.05$). There was a significant difference in monoculture maize and soybean rhizosphere bacterial community structures among the five stages and in those of intercropping soybean and maize at the S1(S2), S3, S4, and S5 stages. The monoculture and intercropping soybean rhizosphere bacterial community structure was significantly different at the S1, S2 and S4 stages, and the monoculture and intercropping maize rhizosphere bacterial community structure was significantly different at the S2 and S3 stages.

Changes in bacterial taxa in rhizosphere soils at five different stages

The soil bacterial communities were significantly altered during the five different growth stages. The phyla *Actinobacteria, Proteobacteria, Chloroflexi, Firmicutes, Acidobacteria,* and *Bacteroidetes* accounted for 88.02 and 96.38% of those communities in soil samples (Fig. 4). The relative abundance of the top 25 genera with significant difference in the soil is shown in Table S4. The relative abundance of *Sphingomonas* and *Nocardioides* in intercropping soybean in the S1 stage was significantly higher than that of monoculture soybean, and there were no significant differences in the abundance of other species. The relative abundance of *Chryseobacterium*, and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* in intercropping soybean in the S2 stage was significantly higher than that in monoculture soybean, and the relative abundances of *Bradyrhizobium*, *Intrasporangium*, *Enterobacter*, *Acinetobacter*, *Microbacterium*, Uncultured bacterium, *Mycobacterium*, and *Ktedonobacter* were significantly lower than those of monoculture soybean. The relative abundance of *Bacillus*, *Fictibacillus* and *Oryzihumus* in intercropping maize was significantly higher than that of monoculture maize. *Metagenome*, uncultured *Acidobacteria bacterium*, *Burkholderia-Caballeronia-Paraburkholderia*, and *Bryobacter* were significantly lower in abundance in intercropping than in monoculture maize, while there was no significant difference

in other genera. In the S3 period, the relative abundance of *Bacillus* and *Mesorhizobium* in intercropping soybean was significantly higher than that of monoculture soybean. *Enterobacter* and *Sphingobacterium* showed a significantly lower relative abundance in intercropping than in monoculture soybean. The relative abundance of *Streptomyces* in intercropping maize was significantly lower than that in monoculture maize, and there was no significant difference in other genera. The relative abundance of *Bradyrhizobium* in the intercropping soybean samples was significantly higher than that in the monoculture soybean samples in S4. The relative abundances of *Intrasporangium*, *Metagenome*, *Acinetobacter* and *Oryzihumus* were significantly lower in intercropping soybean than in monoculture soybean, and the relative abundance of *Streptomyces* was significantly lower than that in monoculture maize, with no other significant differences. During the S5 period, the relative abundance of *Enterobacter* microbes in intercropping soybean was significantly lower than that in monoculture soybean, while that of *Metagenome* and *Luedemannella* was significantly lower than that of monoculture maize, and there were no other significant differences.

The relationship between physicochemical properties and the bacterial community

The mantel test was performed to examine the correlation between physicochemical properties and bacterial community composition. The results showed that pH and SOM were significantly correlated with the rhizosphere soil bacterial communities ($P < 0.05$, Table 2). The SOM had the highest correlation with the rhizosphere soil bacterial community (Bray-Curtis distance, $r = 0.2756$, $P = 0.001$), with no significant correlation found between AP, AN, AK, ACd and the rhizosphere soil microbial community. To determine the relative contribution of environmental variables to the bacterial community, canonical correspondence analysis (CCA) and CCA-based variation partitioning analysis (VPA) were further performed. CCA-based VPA showed that pH, SOM, ACd, and available (P, K, N) explained 2.82%, 3.43%, 1.34% and 5.43% of the variation in the rhizosphere soil bacterial community, respectively. Their interaction could explain 3.02% of the variation, leaving 83.96% of the variation unexplained (Fig. 5).

Discussion

Soil microorganisms participate in many ecological processes in nature and have a great influence on soil quality and function (Rovira, 1965). In our study, we analyzed the contents of pH, SOM, AN, AP, AK, and ACd in the soil of intercropping soybean-maize systems at different growth stages. AN, AP and AK refer to the N, P, and K in the soil that is easily absorbed and utilized by crops. Different planting patterns will change the soil nutrient composition of the crop. Previous studies have shown that intercropping enhances soil carbon and nitrogen (Cong et al., 2015). The maize-peanut intercropping improved levels of soil nutrients (available nitrogen and phosphorus) and enzymes activities (Li et al., 2018). And Fu et al (2019) also found that the soil total nitrogen, AP and SOM contents in the intercropping crop soil samples were significantly higher than corresponding monoculture soil samples. In our study, the AK content of the intercropping soybean soil samples was higher than the corresponding monoculture soybean soil samples. The AP content in the intercropping soybean/maize soil samples was higher than the

corresponding monoculture soil samples at five different growth stages, which indicated that the soybean-maize intercropping pattern also increased the AP content in the soil.

In addition, the soil pH of the monoculture soybean rhizosphere samples decreased, while that of the maize samples increased, indicating differences in rhizosphere soils among different species and growth stages. The pH of intercropping soybean and maize rhizosphere soil samples all decreased and then increased, showing the differences in rhizosphere soils at different growth stages (Fig. 1). Previous studies showed that pH and organic matter were both important factors affecting crop growth and soil microorganisms (Jung et al., 2008; Wang et al., 2019). In our study, the mantel results also indicated that pH and organic matter were the key factors affecting crop rhizosphere soil microorganisms (Mantel, $P<0.05$).

The content of ACd in monoculture soybean rhizosphere samples all decreased and then increased, but the intercropping soybean soil samples indicated an opposite trend. The dynamic trend of the physicochemical properties of maize rhizosphere soil was opposite to that of soybean rhizosphere soil (Fig. 1). Interestingly, we found that the ACd content in intercropping soybean and maize soil was significantly different from the corresponding monoculture crop soil since the S2 stage. With the growth of the two crops, the difference in the ACd content between monoculture and intercropping soybean/maize soil gradually decreased. The results showed that there was a significant difference in the absorption of ACd between intercropping and monoculture planting patterns. The intercropping planting pattern changed the absorption of ACd in the maize and soybean soil since the S2 stage and showed a different change in different crop stages. The results showed that the cadmium content in cadmium-polluted soil could be altered by the soybean/maize intercropping system (Li et al., 2008).

Plants release chemicals to the surrounding environment by allelopathy to affect other plants and microbes (Inderjit and Jacob, 2001). Allelopathy produced by secondary substances between different intercropping crops is widespread in nature, and these allelochemicals may also directly or indirectly affect the soil microbial community structure, number, composition and diversity (Inderjit and Jacob, 2001).The dominant phyla in the soybean and maize rhizosphere soil samples were *Proteobacteria, Chloroflexi, Acidobacteria, Actinobacteria* and *Firmicutes*, which is similar to results reported in other agricultural crops. The main phyla were similar among the rhizosphere of the same crop in monculture cropping and intercropping systems, but there was a slight difference among the different growth stages in our study.

A significant difference in the main phyla was observed between soybean and maize rhizosphere soil samples, which resulted from the crop species genotype (Correa et al., 2010; Sapkota et al., 2015). When comparing the bacterial community diversity of soybean and maize under the two planting modes, we also found that the same crop showed little difference under the two modes (Yong et al., 2012). However, the bacterial community diversity of maize rhizosphere soil was significantly higher than that of soybean rhizosphere soil. The results showed that maize was enriched with more bacteria than soybean, which changed the absorption of ACd in maize. Previous research indicates that intercropping planting patterns

not only lead to significant changes in microbial diversity but also change the microbial community composition and function (Sun et al., 2009). We found a slight difference in bacterial diversity. In our study, microbial communities of soil samples from different growth stages were significantly different, indicating the significance of crop growth stages in microbial community changes. The bacterial communities of intercropping and corresponding monoculture soybean were significantly different at S1, S2 and S4, while those of maize were significantly different at the S2 and S3 growth stages. This result indicated that the crop rhizosphere bacterial community is not only regulated by the planting pattern but also influenced by the crop growth stage. These microorganisms also regulate the rhizosphere environment by plant-microbe interactions (Hu et al., 2020). Regulating the cadmium content in cadmium-contaminated soil through plant-microbial interactions will be our next research focus.

Abbreviations

SOM: soil organic matter; AP: available phosphorus; ACd: available cadmium; AN: available nitrogen; AK: Available potassium; pCoA: the principal coordinate analysis; ADONIS: non-parametric permutational multivariate analysis of variance of the Adonis function. OTU: operational taxonomic unit; PERMANOVA: nonparametric permutational multivariate

Declarations

Acknowledgements

The authors are thankful to the Crop Research Institute of Hunan Province for their support in this experiment.

Authors' contributions

HL and LY L designed the experiments, BY, HL G, ZY C, QZ and SL C performed the experiments, HL, and LY L analyzed the data, HL prepared figures and/or table, LY L and ZH C revised this manuscript language. All authors read and approved the final manuscript.

Funding

This work was supported by the National Key Research and Development Program of China 2016YFD0300109; 2016YFD030020907;the China Agriculture Research System (No.CARS-02); the Natural Science Foundation of Hunan Province2018JJ3320.

Availability of data and materials

All data obtained have been included into the manuscript and its additional files.

Ethics approval and consent participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

1 Crop Research Institute, Hunan Academy of Agricultural Sciences, Changsha, China; 2 Yangtze Normal University, Chongqin, China

References

Anderson MJ (2010) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Berendsen R L, Pieterse C M, Bakker P A (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17(8): 478-486. https://10.1016/j.tplants.2012.04.001

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336. https://doi.org/10.1038/nmeth.f.303

Chen P, Du Q, Liu X M, Zhou L, Hussain S, Lei L, Song C, Wang X C, Liu W G, Yang F, Shu K, Liu J, Du J B, Yang W Y, Yong T W (2017) Effects of reduced nitrogen inputs on crop yield and nitrogen use efficiency in a long-term maize-soybean relay strip intercropping system. PLoS One 12, e0184503. https://10.1371/journal.pone.0184503

Cong W F, Hoffland E, Li L, Six J, Sun JH, Bao XG, Zhang FS, Van Der Werf W (2015) Intercropping enhances soil carbon and nitrogen. Global Change Biol 21(4):1715-1726. https://10.1111/gcb.12738

Correa O S, Romero A M, Montecchia M S, Soria M A (2010) Tomato genotype and Azospirillum inoculation modulate the changes in bacterial communities associated with roots and leaves. J Appl Microbiol 102(3):781-786. https://10.1111/j.1365-2672.2006.03122.x

Coskun D, Britto D T, Shi W, Kronzucker H J (2017) How plant root exudates shape the nitrogen cycle. Trends Plant Sci 22: 661–673. https://10.1016/j.tplants.2017.05.004

Du J B, Han T F, Gai J Y, Yong T W, Sun X, Wang X C, Yang F, Liu J, Shu K, Liu W G, Yang W Y (2018) Maize-soybean strip intercropping: Achieved a balance between high productivity and sustainability. J of Integrative Agriculture 17:747–754. https://10.1016/S2095-3119(17)61789-1

Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 10:996–998. https://doi.org/10.1093/bioinformatics/btr507

Fehr, W R and Caviness, C E (1977) Stages of soybean development. Special Report 80, Iowa Agricultural Experiment Station, Iowa Cooperative External Series, Iowa State University, Ames.

Harichová J, Karelová E, Pangallo D, Ferianc P (2012) Structure analysis of bacterial community and their heavy-metal resistance determinants in the heavy-metal-contaminated soil sample. Biologia 67,1038–1048. https://doi.org/10.2478/s11756-012-0123-9.

He Y, Ding N, Shi J C, Wu M, Liao H, Xu J M (2013) Profiling of microbial PLFAs: Implications for interspecific interactions due to intercropping which increase phosphorus uptake in phosphorus limited acidic soils. Soil Biol. Biochem 57: 625–634. https://10.1016/j.soilbio.2012.07.027

Hu J, Wei Z, Kowalchalchuk G, Xu Y C, Shen Q R, Jousset A (2020) Rhizosphere microbiome functional diversity and pathogen invasion resistance build up during plant development. Environ Microbiol, 2020. https://10.1111/1462-2920.15097

Hurdebise Q, Tarayre C, Fischer C, Colinet G, Hilligsmann S, Delvigne F (2015) Determination of zinc, cadmium and lead bioavailability in contaminated soils at the single-cell level by a combination of whole-cell biosensors and flow cytometry. Sensors 15, 8981–8999. https://doi.org/10.3390/s150408981

Inderjit and Jacob W (2001) Plant allelochemical interference or soil chemical ecology? Perspectives in Plant Ecology, Evolution and Systematics, 2001. https://10.1078/1433-8319-00011

Kong X, Jin D C, Jin S L, Wang Z G, Yin H Q, Xu M Y, Deng Y (2018) Responses of bacterial community to dibutyl phthalate pollution in a soil-vegetable ecosystem. J Hazard Mater 353:5 https://10.1016/j.jhazmat.2018.04.015

Jung H, Sohn K D, Neppolian B, Choi H (2008) Effect of soil organic matter (SOM) and soil texture on the fatality of indigenous microorganisms in intergrated ozonation and biodegradation. J Hazard Mater 150(3):809-817. https://10.1016/j.jhazmat.2007.05.032

Kong X, Jin D C, Tai X, Yu H, Duan G L, Yan X L, Pan J G, Song J H, Deng Y (2019) Bioremediation of dibutyl phthalate in a simulated agricultural ecosystem by *Gordonia sp*. strain QH-11 and the microbial ecological effects in soil. Sci Total Environ 691-700. https://10.1016/j.scitotenv.2019.02.385

Li N Y, Li Z A, Ding Y Z, Zou B, Zhuang P (2008) Effects of intercropping different crops with maize on the Cd uptake by maize. Chinese J Appl Ecology 19(6):1369-1373.

Li Q S, Chen J, Wu L K, Luo X M, Li N, Arafat Y, Lin S, Lin W X (2018) Belowground interactions impact the soil bacterial community, soil fertility, and crop yield in maize/peanut intercropping systems. International Journal of Molecular Sciences, 19, 622. https://doi.org/10.3390/ijms19020622

Li X, Sun M, Zhang H, Xu N, Sun G (2016) Use of mulberry–soybean intercropping in salt–alkali soil impacts the diversity of the soil bacterial community. Microb. Biotechnol 9:293–304. https://10.1111/1751-7915.12342

Magoc T, Salzberg S L (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27:2957–2963. https://doi.org/10.1093/bioinformatics/btr507

Manching HC, Balintkurti PJ, Stapleton AE (2014) Southern leaf blight disease severity is correlated with decreased maize leaf epiphytic bacterial species richness and the phyllosphere bacterial diversity decline is enhanced by nitrogen fertilization. Front Plant Sci 5:403. https://doi.org/10.3389/fpls.2014.00403

Mendes R, Garbeva P, Raaijmakers J M (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37:634–663. https://doi.org/10.1111/1574-6976.12028

Ritchie, S.W. and Hanway, J J (1982) How a Corn Plant Develops. Special Report No. 48, Iowa State University of Science and Technology, Cooperative Extension Service.

Rovira, A D (1965) Interactions between plant roots and soil microorganisms. Ann.rev.microbiol 19(1):241-266. https://10.1146/annurev.mi.19.100165.001325

Sapkota R, Knorr K, Jorgensen L N, O'Hanlon K A, Nicolaisen M (2015) Host genotype is an important determinant of the cereal phyllosphere mycobiome. New Phytol 207(4): 1134-1144. https://10.1111/nph.13418

Sun Y M, Zhang N N, Wang E T, Yuan H L, Yang J S, Chen W X (2009) Influence of intercropping and intercropping plus rhizobial inoculation on microbial activity and community composition in rhizosphere of alfalfa (*Medicago sativa L.*) and Siberian wild rye (*Elymus sibiricus L.*). Fems Microbiol Ecol 70(2):62-70. https://10.1111/j.1574-6941.2009.00752.x

Wang C Y, Zhou X, Guo D, Zhao J H, Yan L, Feng G Z, G Q, Yu H, Zhao L P (2019) Soil pH is the primary factor driving the distribution and function of microorganisms in farmland soils in northeastern China. Annals Microbiol 69(13):1461-1473. https://10.1007/s13213-019-01529-9

Xue Y, Xia H, Christie P, Zhang Z, Li L, Tang C (2016) Crop acquisition of phosphorus, iron and zinc from soil in cereal/legume intercropping systems: A critical review. Ann. Bot 3:363. https://10.1093/aob/mcv182

Yang F, Liao D P, Wu X L, Gao R C, Fan Y F, Raza M A, Wang X C, Yong T W, Liu W G, Liu J (2017) Effect of aboveground and belowground interactions on the intercrop yields in maize-soybean relay intercropping systems. Field Crops Research 203:16-23. https://10.1016/j.fcr.2016.12.007

Yang Z, Yang W, Li S, Hao J, Su Z, Sun M, Gao Z, Zhang C (2016) Variation of bacterial community diversity in rhizosphere soil of sole-cropped versus intercropped wheat field after harvest. PLoS One 11: e0150618. https://10.1371/journal.pone.0150618

Yin C, Fan F L, Song A L, Cui P Y, Li T Q, Liang Y C (2015) Denitrification potential under different fertilization regimes is closely coupled with changes in the denitrifying community in a black soil. Appl. Microbiol. Biotechnol 99: 5719–5729. https://10.1007/s00253-015-6461-0

Yong T W, Liu X M, Yang F, Song C, Wang X C, Liu W G, Su B Y, Zhou L, Yang W Y (2015) Characteristics of nitrogen uptake, use and transfer in a wheat-maize-soybean relay intercropping system. Plant Prod. Sci 18, 388–397. https://10.1626/pps.18.388

Yong T W, Yang W Y, Xiang D B, Zhu Z Y (2012) Effect of wheat/maize/soybean and wheat/maize/sweet potato relay strip intercropping on bacterial community diversity of rhizosphere soil and nitrogen uptake of crops. Acta Agronomica Sinica 38(2):333-343. https://10.3724/SP.J.1006.2012.00333

Zaeem M, Nadeem M, Pham T H, Ashiq, W, Thomas, R (2019) The potential of maize-soybean intercropping to improve the soil health status and biomass production in cool climate boreal ecosystems. Sci Rep-UK, 9(1). https://10.1038/s41598-019-49558-3

Zhang X, Huang G, Bian X, Jiang X, Zhao Q (2012) Review of researches on advantages of intercropping. Asian Agric. Res 114:126-132.

Tables

Table 1 The Student t-test analysis between monoculture and intercropped corn and soybean during various crop growth stages. D: the monoculture soybean, Y: monoculture corn, JD: intercropped soybean, JY: intercropped corn. -: no significant difference; * : $p<0.05$; ** : $p<0.01$; *** : $p<0.001$.

		D	JD	p(D-JD)	Y	JY	p(Y-JY)
pH	S1	5.63±0.03	5.6±0	-	5.3±0	5.38±0.03	*
	S2	5.6±0	5.83±0.12	-	5.4±0	5.38±0.03	-
	S3	5.53±0.09	4.78±0.05	***	5.38±0.05	5.18±0.05	*
	S4	5.18±0.03	5.25±0.03	-	5.43±0.08	5.28±0.05	-
	S5	5.15±0.1	5.45±0.03	*	5.6±0	5.75±0.1	-
SOM	S1	28.25±0.4	29.4±0.19	*	31.48±0.33	29.43±0.08	***
	S2	25.25±1.06	30.23±0.72	**	30.5±0.75	30.8±0.16	-
	S3	25.83±0.62	27.68±1.24	-	30.68±0.58	30.53±0.28	-
	S4	32.08±0.44	32.38±1.37	-	28.08±0.44	34.63±0.49	***
	S5	32.75±1.68	33.18±1.35	-	31.03±0.7	32.53±0.64	-
AP	S1	14.55±0.34	20.5±0.88	***	38.38±1.13	89.78±0.33	***
	S2	7.33±0.7	19.08±0.14	***	21.55±0.43	27.9±0.77	***
	S3	16.15±2.19	28.53±0.76	**	20.68±0.2	28.85±3.48	-
	S4	14.05±0.58	28.3±5.52	*	20.9±1.33	27±2	*
	S5	16.98±0.11	27.6±1.05	***	23.6±0.29	26.35±1.02	*
AN	S1	121.5±3.38	118±5.6	-	253.25±4.23	331.25±1.18	***
	S2	107.25±0.75	222±0.58	***	203.5±23.24	247.25±4.07	-
	S3	189±1.22	108.75±0.48	***	161.5±21.15	165.25±21.46	-
	S4	133±3.49	132±8.71	-	137.25±0.48	180.5±12.98	*
	S5	109.75±0.25	138±8.53	*	194.75±1.55	166.5±2.4	***
AK	S1	80.25±0.25	95.75±0.85	***	145.75±0.85	239.75±0.63	***
	S2	59.75±0.25	70.25±0.48	***	91.75±1.38	74.5±3.23	**
	S3	72.75±3.12	99.75±8.23	*	79.25±3.01	53.25±2.02	***
	S4	71.75±1.55	136.5±12.1	**	90.5±7.58	78.25±6.57	-
	S5	90±2.27	127±9.43	**	139.75±0.63	89.5±2.53	***
ACd	S1	0.26±0.01	0.24±0.03	-	0.18±0.03	0.12±0.04	-
	S2	0.05±0.02	0.3±0	***	0.48±0.26	0.06±0.02	-
	S3	0.06±0.01	0.22±0.06	*	0.37±0.07	0.07±0	**
	S4	0.07±0.02	0.16±0.02	*	0.17±0	0.34±0.07	*
	S5	0.19±0.03	0.09±0	*	0.22±0	0.2±0.01	-

Table 2 Mantel analysis of the relationship between the microbial community structure and soil properties. P-values were calculated using the distribution of the Mantel test statistics estimated from 9999 permutations. *: P<0.05, **: P<0.01, ***: P<0.001.

Soil properties	Bray–Curtis		Jaccard	
	r	p	r	p
pH	0.1344	0.037*	0.1456	0.024*
SOM	0.2756	0.001***	0.293	0.001***
AP	-0.0505	0.72	-0.0271	0.585
AN	-0.0366	0.699	-0.0237	0.619
AK	-0.0783	0.879	-0.0522	0.766
ACd	-0.0319	0.573	-0.0431	0.695

Figures

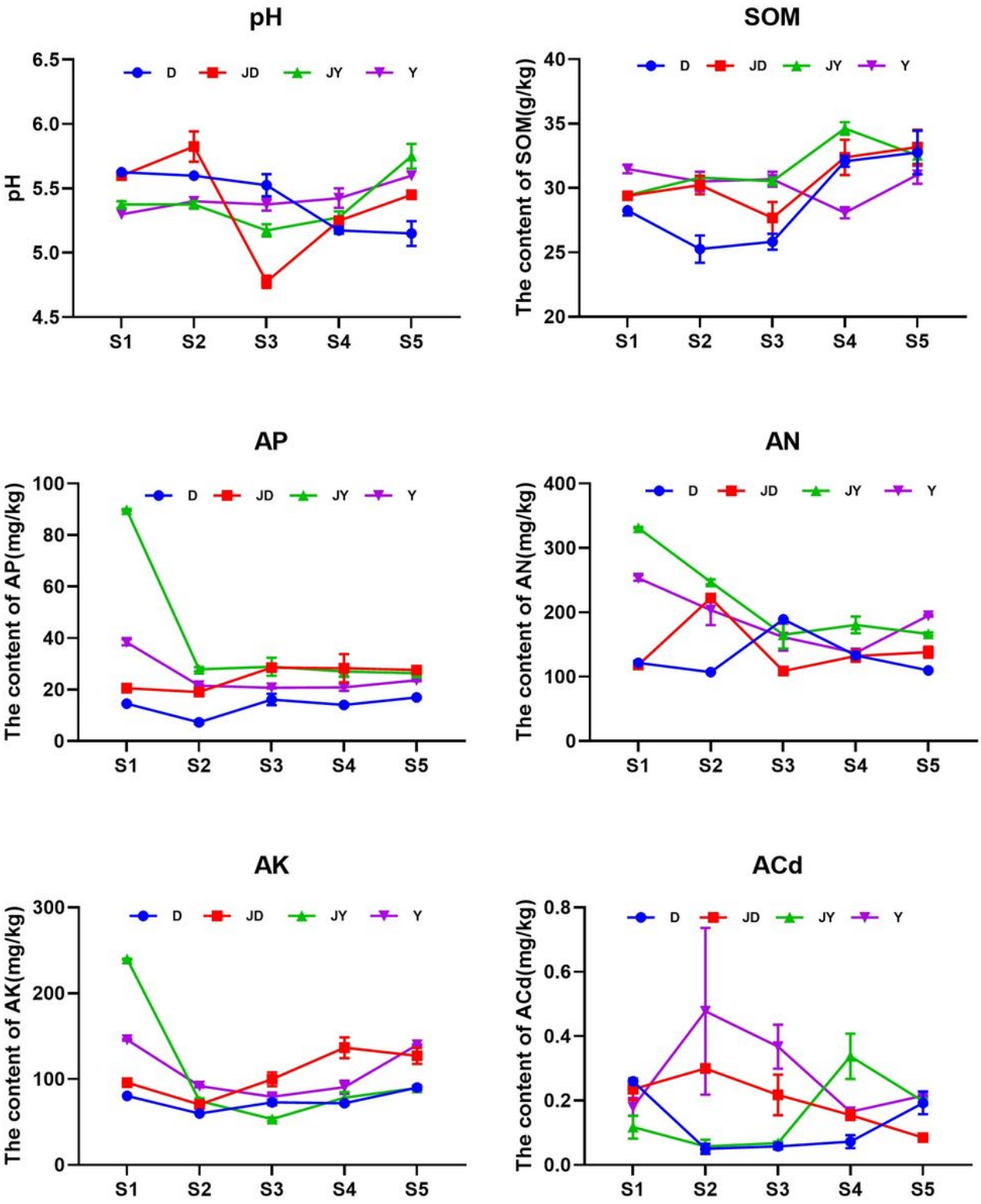

Figure 1

Physicochemical properties of different rhizosphere soils. SOM: Soil organic matter, AP: available P, AN: available N, AK: available K, ACd: available Cd. D: the monoculture soybean, Y: monoculture corn, JD: intercropped soybean, JY: intercropped corn. S1-S5:V2, V6, R4, R6, R8 for soybean and V3, V6, VT, R3, R6 for maize.

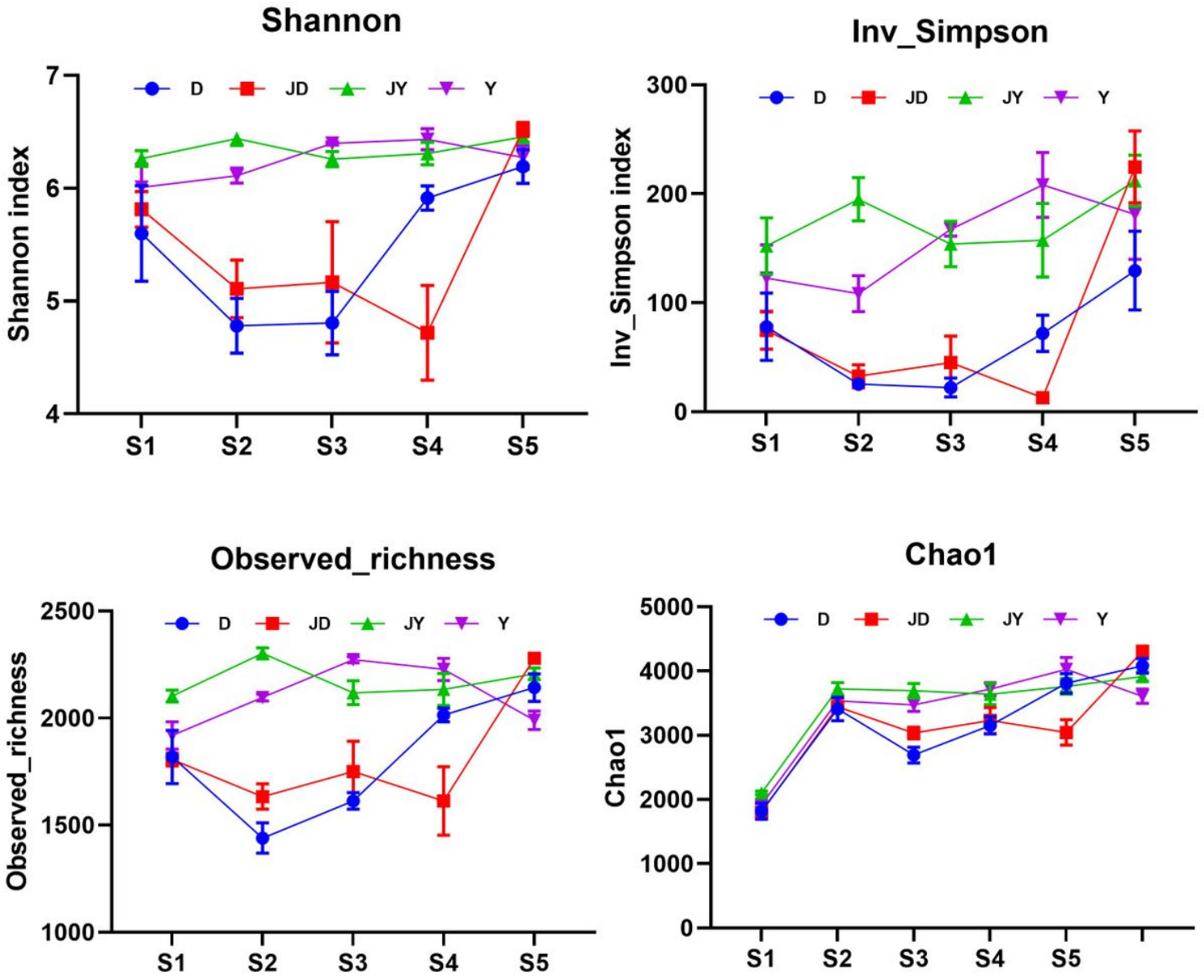

Figure 2

The α diversity of different rhizosphere soils. S1-S5:V2, V6, R4, R6, R8 for soybean and V3, V6, VT, R3, R6 for maize.

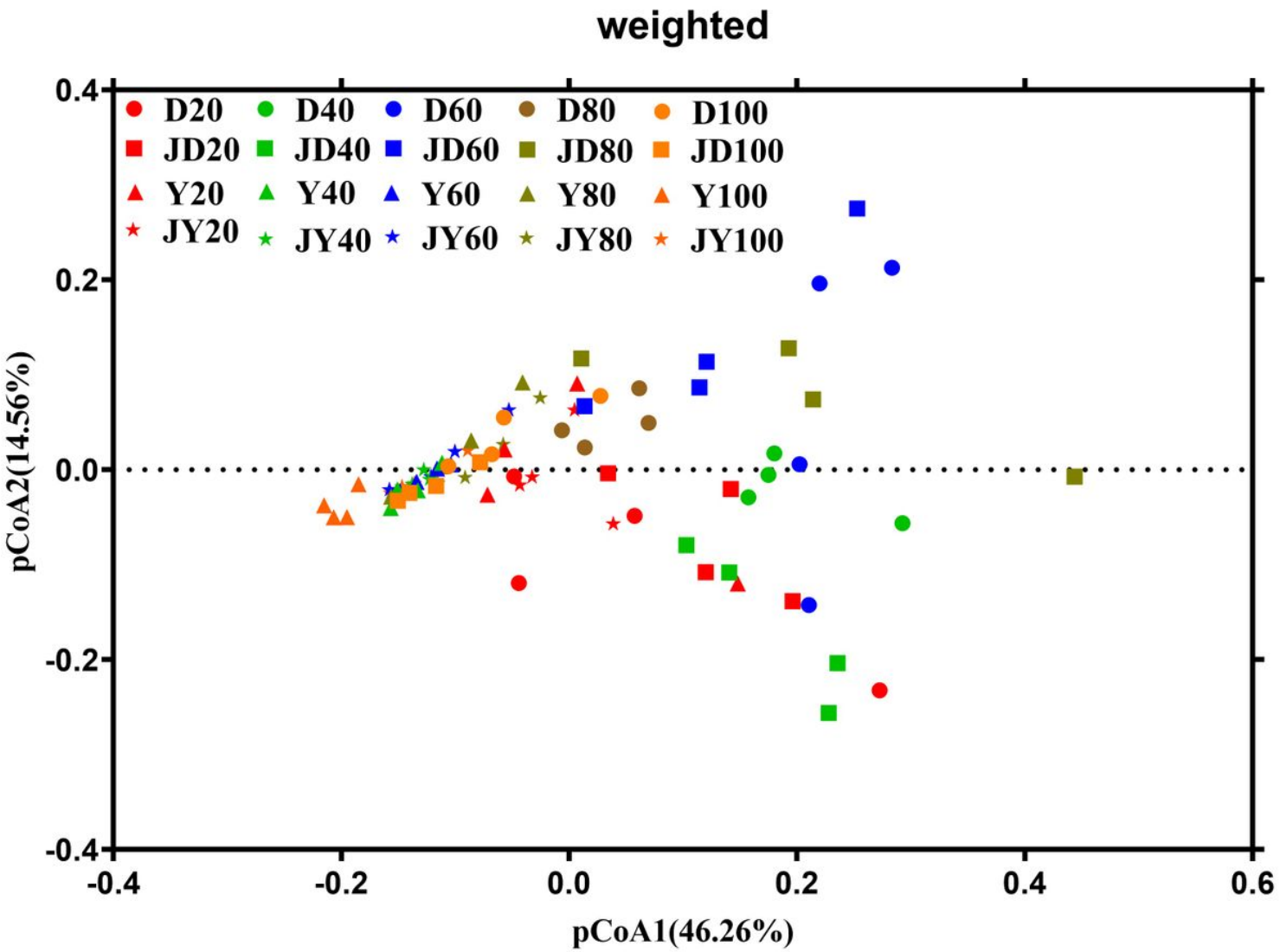

Figure 3

Principal coordinate analysis (weighted_PCoA) of microbial communities based on Bray-Curtis dissimilarity matrices. D20, D40, D60, D80, D100: the five growth stages of monoculture soybean; JD20, JD40, JD60, JD80, JD100: the five growth stages of intercropping soybean; Y20, Y40, Y60, Y80, Y100: the five growth stages of monoculture corn; JY20, JY40, JY60, JY 80, JY 100: the five growth stages of intercropping soybean.

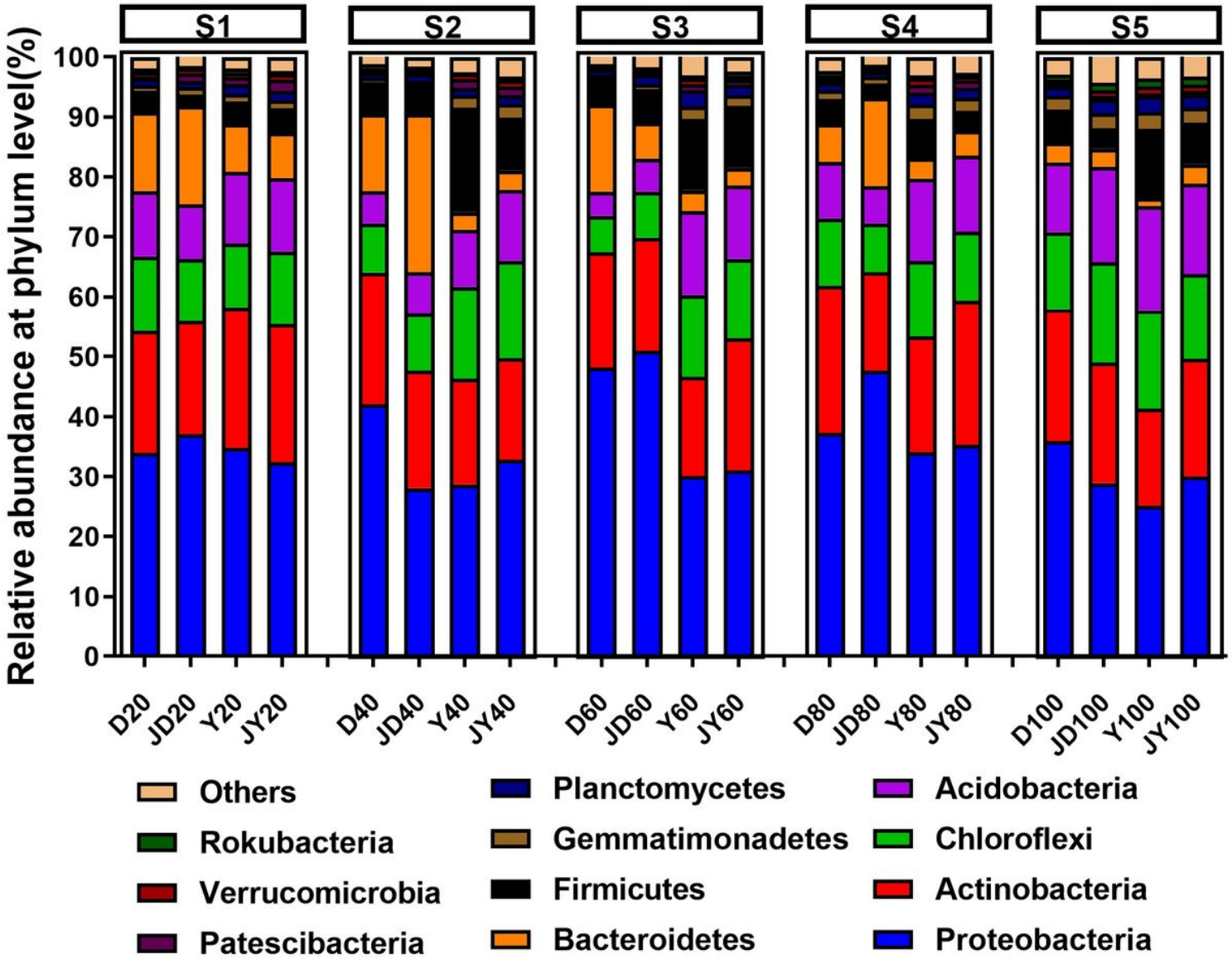

Figure 4

The relative abundance of the dominant phyla in the different rhizosphere soils. D: the monoculture soybean, Y: monoculture corn, JD: intercropped soybean, JY: intercropped corn. S1-S5:V2, V6, R4, R6, R8 for soybean and V3, V6, VT, R3, R6 for maize.

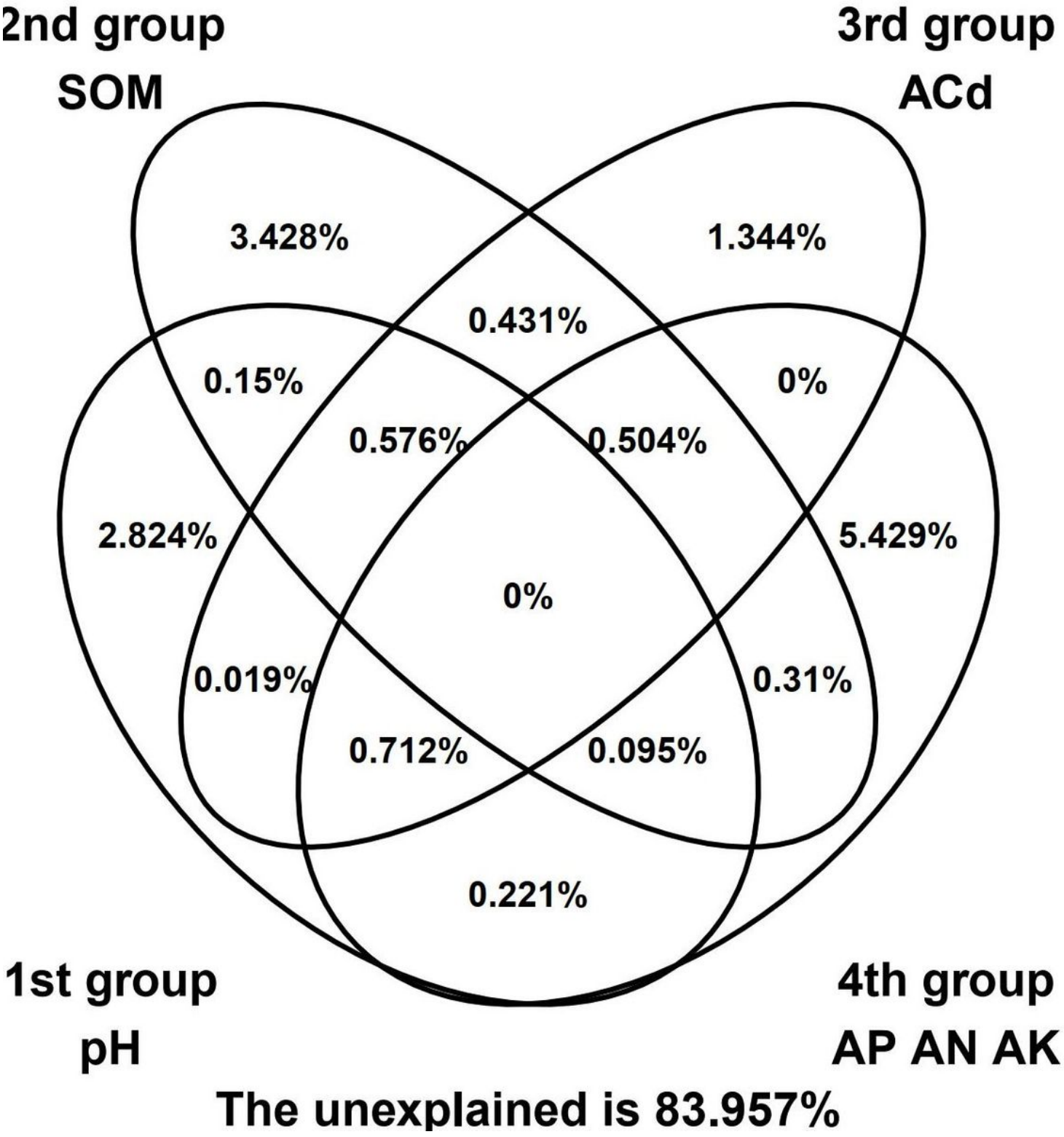

Figure 5

The variation partitioning analysis (VPA) based on the canonical correspondence ananlysis. SOM: Soil organic matter, AP: available P, AN: available N, AK: available K, ACd available Cd.

Supplementary Files

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

- SupplementTables.docx