

The response of sugar beet rhizosphere micro-ecological environment to continuous cropping

Rufei Cui

Gui Geng

Gang Wang

Piergiorgio Stevanato

Yinzhuang Dong

Tai Li

Lihua Yu

Yuguang Wang (✉ wangyuguang@hlju.edu.cn)

Heilongjiang University <https://orcid.org/0000-0003-2260-5652>

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Abstract

Background and aims Continuous cropping can lead to increased soil-borne diseases of sugar beet and cause economic losses. We aimed to study the relationship of continuous cropping to the microbial community of each root compartment of sugar beet (*Beta vulgaris* L.).

Methods The structure and difference of microbiome community in bulk soil (Sc and Sn), rhizosphere soil (Rc and Rn), and endosphere (Bc and Bn) of sugar beet were analyzed by high-throughput sequencing technique.

Results The endophytic microbial alpha diversity of the root system was significantly lower in the continuous crop group than in the non-continuous crop group. The relative abundance of *Proteobacteria* increased and the relative abundance of *Actinomycetes* decreased in continuous cropping compared to non-continuous cropping. The relative abundance of *Tausonia* and *Fusarium* in each group of continuous cropping is greater than the non-continuous cropping. Microbial communities in all three root compartments were correlated with environmental factors, except for the rhizosphere bacterial community. Root compartments in continuous cropping are dominated by pathogenic fungi. The structural characteristics of the fungal zone show a continuous and progressive change from the bulk soil to the rhizosphere soil and then to the endosphere of sugar beet.

Conclusions The accumulation or subtraction of some microbial populations during continuous sugar beet cultivation to form a specific flora has proven to be an important strategy for building the micro-ecological community structure of sugar beet. This provides a scientific reference for understanding the formation of continuous cropping obstacles in sugar beet.

Introduction

Sugar beet (*Beta vulgaris* L.) is not only the raw material in the sugar industry but also an important global feed (Chhikara et al. 2019; Li et al. 2020). The planting area accounts for about one-third of the global sugar crop planting area and provides 16% of the world's sugar production (de Oliveira et al. 2020; Geng and Yang 2015). The cultivation area and yield of sugar beet are second only to sugar cane (Mall et al. 2021). A good soil environment is a prerequisite for high sugar yields in sugar beet, which is a taproot crop that should not be grown continuously (Geng et al. 2020; Huang et al. 2021). However, due to poor land use and cultivation practices, sugar beet is often grown in succession resulting in poor plant growth, frequent pests and diseases, reduced sugar content and lower yields (Huang et al. 2020b).

Soil and crops harbor an extraordinarily rich diversity of microbiome that can impact their health by influencing the nutrient element cycle and affecting soil fertility (Bai et al. 2015b; Zhu et al. 2020a). Soil microorganisms play key roles in the cycle of soil nutrient elements and soil material metabolism but are sensitive to changes in environmental conditions (Bastida et al. 2021). They are affected by soil properties (Subhashini and Kumar 2019). Most of these soil properties are associated with microbiomes, soil pH and enzyme activity (Wang et al. 2020). Additionally, the endophytic microbiome has been widely

used as the best indicator of host plant fitness (Zhu et al. 2018). Notably, plant root-associated, bulk, and rhizosphere microbiomes have caught widespread attention owing to their crucial roles in host growth and development (Fitzpatrick et al. 2018).

Black soils play a key role in the crucial soil resources of Northeast China and are one of the most important factors in lasting national food security (Liu et al. 2020b). The intensification of sugar beet monoculture is happening on a wide scale in these areas due to limitations in cultivated land, economic benefits, and a requirement for enhancing regional agricultural industrialization (Samadi et al. 2008). Recent studies have shown that long-term continuous cropping affects the plant root-associated, bulk, and rhizospheric soil microbial structure (Bracho-Mujica et al. 2019; Liu et al. 2020a; Miao et al. 2016; Yuan et al. 2021a). On the contrary, such variations further contribute to the degree of long-term continuous cropping obstacles (Sun et al. 2017). Thus, the healthy and stable microbial community structure in the microecology may be essential for maintaining stable crop yields and relieving continuous cropping obstacles (Gao et al. 2019). It has recently been found that continuous cropping of sugar beet can lead to changes in soil chemical properties (Perez-Brandan et al. 2014), alterations in soil enzyme activity, soil-borne pathogen accumulation (Xiong et al. 2015), enrichment of allelochemical substances, and soil microbial community changes (Bai et al. 2015a). Therefore, understanding how plant root-associated, bulk, and rhizosphere microbiomes respond to agricultural management measures and crop physiological conditions are of great significance to agricultural production (Chen et al. 2019).

The effects of continuous crop barriers on the soil micro-ecological environment and plant growth and the links between them are not clear. And there are no independent studies on the diversity of bulk soil, rhizosphere soil, and endophytic microbial communities during the continuous growth of sugar beet seedlings. Therefore, this research applied high-throughput sequencing technology to analyze changes in microbial community structure in the micro-ecological environment of sugar beet in continuous cropping fields.

In the present study, we used pot experiments to examine the microbial composition and functions during sugar beet rotation and continuous cropping systems in Northeast China. This study collected sugar beet seedling roots, rhizosphere soil, and bulk soil for microbial community sequencing analysis. The objectives of this study were (1) to reveal the bacterial and fungal community composition of successive sugar beet seedlings, (2) to compare the microbial community composition and evolution in the three root compartments, and (3) to explore potential pathogenic and beneficial microbial changes in successive sugar beet crops. This study aims to provide a theoretical basis for mitigating succession barriers in sugar beet and to provide important guidance for developing improved agricultural regulatory strategies.

Materials And Methods

Plant Material and Experimental Design

Seeds of sugar beet (KWS1176 from KWS Company of Germany) were selected as plant materials in this experiment. In our previous studies, it was found to exhibit strong resistance to adversity and is more

widely used in Northeast China. The soil was sourced from the black soil area in Hulan District, Harbin City, Heilongjiang Province (latitude and longitude: 46°00'14" E, 126°38'49" N). Soil samples included soils from a maize-beet rotation and soils from two years of continuous sugar beet crop, both collected from adjacent plots with similar agricultural management practices. The seeds were sown in a plastic pot containing 0.7 kg of soil. In this experiment, each pot was sown with six seeds and was irrigated with 50 mL of Hoagland nutrient solutions every 10 days. The control group was planted with non-continuous cropping soil, and the continuous planting group was planted with continuous cropping soil. To ensure the normal growth of plant, only one seedling was selected in each pot after five days of planting. All pots were incubated in a laboratory, with the photoperiod: day (lights on) 7 am–9 pm, 24 °C; night (lights off) 19 °C. Thirty pots were set up for each treatment and each pot was considered a biological replicate.

Plant and soil sampling

Harvesting was carried out on 30 days. Beetroots, rhizosphere soils, and bulk soils were collected by the method described by Bulgarelli et al (Bulgarelli et al. 2012). The beet was randomly selected at each treatment group for uniform growth, each treatment consisted of 5 replicates, and samples from 3 pots were selected for mixing and recorded as one replicate. Beet plants were manually collected from the plastic pots and bulk soil aggregates were separated by shaking plant roots. The bulk soil samples were sampled from the pots and were passed through the sterile 2-mm sieve. The bulk soil is divided into two parts for subsequent analysis. One portion was air-dried for soil properties and soil enzyme activities, and the other portion was frozen in liquid nitrogen and stored at -80°C for the sequencing of microbes. To standardize the sampling, we used a sterile scalpel to dissect the roots under the beet seedling stems to maximize repeatability. Root sections of plants were pooled into a sterile 50 ml tube containing 25 mL sterile Silwet L-77 amended phosphate-buffered saline solution (PBS, per liter: 7 mM Na₂HPO₄, 3 mM NaH₂PO₄, 200 µL Silwet L-77, pH 7.0) and were vigorously mixed with a vortex for 15 s to detach from the root surfaces to produce the rhizosphere soil. Then samples were subjected to centrifugation at 3200 ×g for 15 min to get the rhizosphere. For root endosphere metagenomic DNA extraction, roots were transferred to a new 50 ml sterile tube containing 25ml PBS-S, and vortexed. Repeat the steps until the phosphate buffer in the centrifuge tube is no longer turbid. Then, the root was transferred into another sterile tube and insonated at low frequency for 10 cycles, consisting of 30-second bursts and rests of 30 seconds. The purpose of this step can remove the microorganisms attached and further clean the outer surface of the roots. Finally, the roots were transferred to a fresh volume of 25 mL PBS-S and sonicated roots were defined as the root endosphere. Each root endosphere sample was then stored at -80°C until DNA extraction. Through the use of scanning electron microscopy and microbial culture techniques, Xiao et al. (Xiao et al. 2017) confirmed that the microorganisms on the root surface could be removed by the above steps.

Determination of Soil Properties and Plant indicators

Three sugar beet plants of uniform growth were randomly selected from each treatment group and washed with deionized water for measuring root vigour. The root vitality of the sugar beet plants was

measured according to the alpha naphthylamine method (Ramasamy et al. 1997). Root morphology and root area were determined by WINRHIZO software (Regent Instruments Inc., Quebec, Qc, Canada). The air-dried bulk soil samples were passed through a 2-mm sieve as described by Bao (Bao 2005). Air-dried soils are used to determine soil physicochemical properties and soil enzyme activity (pH, EC, AN, AK, AP, sucrase, acid phosphatase, CAT, and urease). Specifically, the hydrogen potential (pH) and electrical conductivity (EC) of the soil were measured with a potentiometer after shaking for 30 min at the soil to distilled water ratio of 1:2.5. Soil available nitrogen (AN) was measured by changing available nitrogen into ammonia. Soil available phosphorus (AP) was determined by leaching with sodium bicarbonate solution and molybdenum-antimony colorimetric method. Soil available potassium (AK, extracted in ammonium acetate) was determined by the flame photometer. Soil catalase activity (CAT) was measured according to KMnO_4 titration. Soil urease is based on urea and the enzyme activity is determined based on the enzymatic product ammonia interacting with phenol-sodium hypochlorite to produce blue indophenol. Soil sucrase content was determined by a colorimetric method using 3,5-dinitroso salicylic colorimetry and the amount of reducing sugars was used to express the enzyme activity. Soil acid phosphatase activity was determined by a colorimetric method using sodium benzene phosphate to express enzyme activity as phenol content. All bulk soil samples were assayed in five replicates and inorganic controls were required for each treatment for the soil enzyme assay.

DNA Extraction and DNA sequencing

The non-continuous cropping sugar beet bulk soil (Sn), its rhizosphere soil (Rn), its endophytic (Bn), and continuous cropping samples (Sc, Rc, and Bc) were extracted as research materials. DNA was extracted from bulk soil, inter-rhizosphere soil and root samples (0.5 g per sample) by the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.). DNA quality and integrity were examined using 1% agarose gel electrophoresis. 16S rRNA genes were selected for the V3-V4 region and fungal ITS amplification was selected for the ITS1-ITS2 region (Huang et al. 2020a). This 16S and ITS high throughput sequencing were performed by BIOZERON Ltd (Shanghai, China).

Processing of Sequencing Data

The MiSeq platform PE250 (Illumina, Inc., CA, USA) was used for double-end sequencing and the PANDAseq software was used for splicing to obtain long reads with highly variable regions. The raw sequence data were analyzed using QIIME, and the 250bp reads were truncated receiving a low-quality score (≤ 20), discarding them were shorter than 50bp (Caporaso et al. 2010). Removal of chimeras using UCHIME software (Edgar et al. 2011). OTU clustering based on 97% sequence similarity using the UPARSE plug-in (Edgar 2010). Species annotation of OTU representative sequences against the Greengenes database was performed using the RDP classifier (80% confidence interval) (Wang et al. 2007). OTUs with annotated results for chloroplasts and mitochondria or OTUs with only one sequence (singletons) were removed. Given the variation in sequencing depth between samples, the OTUs were normalised for subsequent analysis using the least square method.

Statistical Analyses

The rarefaction was calculated using the software Mothur (Schloss et al. 2009) and conducted to reveal the alpha diversity, including the Chao 1, ACE, Richness, and Shannon indices (Schloss et al. 2011). To test the statistical significance of the structural similarity between communities with different sampling treatments, UniFrac-based hierarchical clustering (Lozupone et al. 2011) was performed using the community ecology package (Wang et al. 2012). Displacement multivariate analysis of variance (PERMANOVA) and non-metric multidimensional scaling analysis (NMDS) were used to examine and visualise differences in the structure of the bacterial and fungal communities at the three root chambers, respectively. To identify biomarkers of important microbial taxa, LEfSe (linear discriminant analysis effect size) analysis was performed as previously reported (Segata et al. 2011). The relationship between changes and dissimilarities was analyzed by the Kruskal-Wallis sum-rank test, and the threshold was set at 0.05. LDA analysis was set as the threshold for the size effect of each OTUs abundant taxa (Ijaz et al. 2018). To further evaluate the relationship between microbial community composition and nine factors of physicochemical properties, the Spearman rank correlation and Mantel tests were visualized using the "ggcor" software package (Yuan et al. 2021b). Functional annotation of 16S rRNA bacterial gene sequences from the SILVA database was performed using the Tax4Fun software package and the FAPROTAX package. Analysis of variance, Tukey's test, and Duncan's test were performed using IBM SPSS 19.0 software. Box line plots were drawn using PRISM (Version 7.0) software. The rest of our analyses were performed with the aid of R software (Version 4.0.0) and the BIOZERON cloud platform (<http://www.cloud.biomicroclass.com/CloudPlatform/home>).

Accession number

The original read registration numbers are PRJNA793140 (16S data) and PRJNA793139 (ITS data), located in the NCBI SRA database.

Results

Soil properties and plant growth indicators

The continuous cropping group (C) decreased soil pH and AK compared with the non-continuous cropping (N). EC, AP, and AN indicators were much higher in the C group relative to the N group (Fig. 1a-e). In addition, the C soil exhibited much higher catalase, and urease contents, while the N group had higher sucrase, and acid phosphatase contents (Fig. 2f-i). We found that the plant height (Height), root dry (DW), root vitality (RA), and root surface area (RSA) in site with the C group were significantly lower than the group with N (Fig. 2j-m, Fig. S1).

Alpha diversity of the microbial community

Alpha diversity of the bacterial and communities in these root compartment samples showed different OTU richness at 97% sequence similarity level. We evaluated the alpha diversity of different root

compartments using the Chao1, Richness, Shannon, and Simpson indices to measure microbial community diversity and richness, respectively (Fig. 3, Table S4-5). The results showed that the Chao1, Simpson, and Shannon diversity indices of the bacterial communities differed significantly ($p < 0.05$) in the endophytic roots. In addition, the fungal community alpha diversity sample Sc was found to be significantly smaller ($p < 0.05$) than sample Sn (Fig. 2). The results showed that the diversity of the microbial community in the root chamber samples changed after continuous crop, with the continuous crop group being significantly lower than the non-continuous group. It indicated that cropping would affect the alpha diversity in plants and soil.

Beta diversity of the microbial community

For a better estimation of the distance relationship between these root compartment samples, based on the unweighted UniFrac distance matrix, the microbial β diversity was further evaluated. We found that the community composition of the microbiota differed in samples. The NMDS results showed that the OTU distribution of the root samples was quite different from other samples, and the bulk soil and rhizosphere soil were clustered together (Fig. 3c, d). The Spearman clustering heat map shows that endosphere (Bc and Bn) samples are correlated, samples from bulk soil (Sc and Sn) are correlated with samples from rhizosphere soil (Rc and Rn), and importantly bulk soil and rhizosphere soil differed significantly from the endosphere (Fig. 3a). For the fungal communities, a similar pattern was observed and the greatest differences were observed between samples Bn and Sc (Fig. 3b). There was a large difference in beta diversity between the C and N samples. Similar results were also seen for the distribution of groups in the non-metric multidimensional scaling (NMDS) ordination, suggesting that microbial community composition varies considerably across soils (Fig. 3c-d). Moreover, the difference between the two treatment groups in rhizosphere soil and bulk soil samples is smaller than that of the roots. The results from the MNDS ranked clustering (Fig. 3c-d) show that all five root compartment samples in groups C and N show a non-random spatial allocation.

Microbial community structure variations

This study also showed the composition of bacterial and fungal species from phylum to genera level in root endosphere, rhizosphere, and bulk soil samples subjected to the different types of soil treatment alternatives. The paradoxical color stripes in Figs. 4 and 5 of different samples reflect that the rhizosphere soil bacteria had conducted alternative colonization on different root compartments. The proportional abundance of dominant taxa changed under different cropping systems and root chamber conditions.

The operational taxonomic units (OTUs) of the different root compartments bacterial communities belong to 39 phyla, 113 classes, 259 orders, 413 families, and 846 genera. Bacterial community circles of phylum abundances and composition is shown in Fig. 4a. The top 10 bacterial phyla in terms of root compartments abundance included *Proteobacteria*, *Actinobacteria*, *Bacteroidota*, *Acidobacteriota*, *Cyanobacteria*, *Chloroflexi*, *Patescibacteria*, *Gemmatimonadetes*, *Myxococcota*, and *Verrucomicrobiota*. The total of these phyla accounted for more than 98.2% of the bacterial sequences. *Proteobacteria* and

Actinobacteria account for 66% of the bacterial community and are the two largest phyla. The relative abundance of *Proteobacteria* increased in the continuous cropping, respectively, compared to levels in the non-continuous cropping, and the abundance of rhizosphere and endosphere is greater than that of bulk soil (Fig. 4c). Furthermore, the Abundance of *Actinobacteriota* decreased. *Pseudomonas*, *Flavobacterium*, and *Pedobacter* were significantly higher in abundance in the continuous cropping samples. In contrast to bulk soil, the rhizosphere and endosphere harbored a higher portion of *Novosphingobium*.

The fungal OTUs belonged to 7 phyla, 25 orders, 71 families, 163 families and 363 genera in the high-throughput sequencing results. A total of four phyla and one unidentified phylum were identified from the phylum level map in Fig. 3b. The four phyla were *Ascomycota*, *Olpidiomycota*, *Basidiomycota*, and *Mortierellomycota*. *Ascomycota* was dominant in continuous cropping soils, especially BC (84.0%), and *Olpidiomycota* was dominated by Bn (85.8%) and Rn (76.7%). The relative abundance of *Tausonia* and *Fusarium* increased in the continuous cropping, respectively, compared to levels in the non-continuous cropping, the *Tausonia* abundance of rhizosphere and bulk soil is greater than that of endosphere, and the *Fusarium* abundance of endosphere is greater than that of other treatment groups (Fig. 4d). Furthermore, the Abundance of *Olpidium* decreased.

In addition, we built four three-axis ternary plots for the most abundant phyla (Fig. 5). Each color circle represents a phylum level, with each corner of the triangle representing a root compartment sample. The dimension of each circle represents its weighted average. Ternary plots showed that many phyla were present in similar proportions at the three sites (bulk, rhizosphere, and endophytic) but that some were comparatively more abundant at a specific position (Fig. 5). In the microbial species, a larger number of phyla showed a corporation with either the bulk soils or rhizosphere soils of the root compartments than with the endophytic. The ternary plots of dominant phyla revealed that different root compartments contained special phyla (Fig. 5). The distribution of other phyla of bacteria in each root compartment is not much different. Furthermore, *Verrucomicrobiota* was more prevalent in endophytic (Fig. 5a-b). *Ascomycota* was abundant in the root compartments of Bc and Sn and *Olpidiomycota* was enriched in the rhizosphere and endophytic of the continuous cropping group (Fig. 5c-d).

Discovery of biomarkers in microbial communities

Differences in root compartments microorganism community were assessed using linear discriminant analysis effect size (LEfSe) analysis at a linear discriminate analysis (LDA) threshold of 3 (Fig. 6). Across the bacterial community, there were more species of *Actinobacteria*, *Acidobacteria*, *Bacteroides* and *Proteobacteria* and there were species differences between the six test groups (Fig. 6a). For root endophytic microbial communities, Bc biomarkers include *Flavobacterium* (its phylum to genus), *Streptomyces* (its order to genus), *Rhizobium* (its order to genus), *Comamonadaceae* (family), *Pseudoxanthomonas* (genus) and *Stenotrophomonas* (genus) and *Alphaproteobacteria* (order) were significantly enriched in Bn. For the rhizosphere microbial community, Rc biomarkers included *Massilia* (its order, family and genus), *Pseudomonas* (its order to genus), *Pedobacter* (by order to genus), *Xanthomonadaceae* (its order and family) and *Proteobacteria* (family), while the Rn biomarkers mainly

include *Novosphingobium* (its order to genus) and *Burkholderia* (its order to genus). In the bulk soil microbial community, *Gemmatimonadaceae* (its phylum to family), *Intrasporangiaceae* (its phylum to family), *Frankiales* (order), *Gaiellales* (order), *Micrococcaceae* (family), *Arthrobacter* (genus) and *Pseudarthrobacter* (genus) were significantly enriched in Sc, while *RB41* (its phylum to genus), *Vicinamibacteraceae* (its order to family), *Nocardioideaceae* (its order to family), *Solirubrobacterales* (its order and phylum) *Microtrichales* (order)), *MB_A2_108* (order), *Chloroflexia* (its phylum and order) and *KD4_96* (order) are significantly enriched in Sn.

Among the fungal communities, *Ascomycota* and *Basidiomycota* species were more numerous and there were species differences between the five test groups (Fig. 6b). For the root endophytic microbial community, the Phylum level of Bc biomarkers included *Ascomycota* and *Basidiomycota*, while the Genus level included *Alternaria*, *Plectosphaerella*, *Dactylonectria* and *Fusarium*, while *Olpidiomycota* (from phyla to genus) was significantly enriched in Bn. For the rhizosphere microbial community, Rc biomarkers included *Trichocladium* and *Tausonia* (from phylum to genus). In the bulk soil microbial community, *Phoma* (its family and genus), *Pseudombrophila* (by order to genus) and *Gibellulopsis* (genus) were significantly enriched in Sc, while *Chaetomiaceae* (its order and family), *Helotiales* (its class and order) and *Mortierella* (by order to genus) were significantly enriched in Sn.

Environmental drivers of root compartments microbial community composition

The influence of environmental variables was explored by calculating correlations between the microbial community composition and environmental factors (Fig. 7). To conclude which environmental factors cause changes in the composition of the microbial community in the root compartments, we correlated differences in functional community composition with soil properties employing distance correction. Soil pH, catalase, urease, and AK were significantly and negatively correlated with AN, AP, EC, acid phosphatase, and sucrase. As shown in Fig. 7, the environmental factors were highly correlated with both bulk soil and endosphere of the bacterial (Fig. 7a) and fungal (Fig. 7b) microbiome ($P < 0.05$). The fungal microbiome of rhizosphere soil is significantly related to environmental factors ($P < 0.05$). However, the bacterial microbiome only has a significant correlation with EC. This suggests the diversity and composition of the rhizosphere fungal community are more closely related to environmental factors than to bacteria. Besides, environmental factors are significantly related to both functional (based on FAPROTAX annotation) and fungal taxonomic composition of the root compartments microbiome. The bacterial taxonomic composition has a not significant correlation with the soil environmental factors ($P > 0.05$).

Functions of Bacterial Communities

Prediction of microbial ecological function in the FAPROTAX database, the annotated OTU is assigned to 61 predicted functional groups. Nevertheless, in the Kruskal-Wallis test, only 50 groups showed significant differences between the six root compartments ($P < 0.05$). Therefore, they are plotted as a functional heatmap (Fig. 8). Among these function predictions, nitrogen (10 groups), carbon (6 groups), sulfur (3 groups), and manganese (1 group) are involved in the geochemical cycle. In the root

compartments, Sc enhanced the soil's functional advantages of phototrophy, photosynthetic cyanobacteria, oxygenic photoautotrophy, and aliphatic non-methane hydrocarbon degradation. These functions including dark oxidation of sulfur compounds, nitrogen respiration, nitrate respiration, and human pathogens were significantly ($P < 0.05$) enhanced in the rhizosphere soil and root zone of the continuous cropping area compared to the non-continuous cropping area.

Tax4Fun (<http://tax4fun.gobics.de/>) is predicted based on the SILVA database. The Sliva database was used to classify OTUs, and a linear relationship between Sliva classification and prokaryotic classification was constructed in the KEGG database to achieve predictions of microbial community function. In addition, for Pathway, three levels of metabolic pathway information and abundance tables at each level can be obtained by using the Tax4Fun pathway. The first reference pathway that contained genes in the first five of the KEGG pathway analysis was metabolic, environmental information processing, genetic information processing, human diseases, and cellular processes. The number of metabolic pathway genes with the highest content is about 25 times higher than that of the second residual genes (Fig. S2a). The metabolic pathways of the second reference pathway containing the first five genes in KEGG pathway analysis were global and overview maps, carbohydrate metabolism, amino acid metabolism, energy metabolism, and membrane transport (Fig. S2b). The metabolic pathways in which the third reference pathway contains the first five genes in KEGG pathway analysis are metabolic pathways, biosynthesis of secondary metabolites, microbial metabolism in diverse environments, biosynthesis of antibiotics, and carbon metabolism (Fig. S2c).

Discussion

Root compartment microorganisms are affected by environmental factors and are an important role in the plant host and soil (Yu et al. 2021). This experiment performed high-throughput sequencing on the bulk soil (Sc and Sn), rhizosphere soil (Rc and Rn), and endosphere (Bc and Bn) of continuous and non-continuous cropping of sugar beet. From bulk soil to rhizosphere to roots, the alpha diversity of bacteria and fungi decreases. In general, the diversity of bacteria in the root chambers of the continuous cropping group decreased and the diversity of fungi increased. From the perspective of microbial structure and composition, the structural characteristics of the microbial flora in the three root chambers showed continuous and gradual changes, indicating that the key microorganisms were attracted to the appropriate location (Hernandez-Alvarez et al. 2022). The results of sugar beet microflora showed that *Actinomycetes* and *Proteobacteria* are their dominant bacterial phyla. Among them, *Proteus* and *Actinomycetes* account for 66% of the bacterial community and are the two largest phyla. Among *Proteobacteria* phylum, *Pseudomonas* resulted as a recurrently abundant genus across sugar beet samples in agreement with recent studies (Bertoldo et al. 2021; Della Lucia et al. 2021). *Ascomycota*, *Basidiomycota*, *Basidiomycota*, and *Mortierella* are their dominant fungal phyla, and *Ascomycota* is the most abundant fungi in continuous cropping soil. The relative abundance of the dominant genera *Tausonia* and *Fusarium* in continuous cropping of sugar beet is higher than that in rotation. It is speculated that these fungi may be closely related to the growth and development of continuous

cropping sugar beet. In addition, *Fusarium* is a pathogen that causes diseases in many plants (Li et al. 2014).

In the three root compartments, the bacterial community levels of continuous cropping and non-continuous cropping groups existed in similar proportions, but the fungal communities were quite different. In the bacterial community, most of the bacterial phyla are enriched in bulk soil and rhizosphere soil. However, *Verrucommicrobiota* is enriched in endophytes. In the fungal community, the dominant phyla of the two treatments are quite different. *Ascomycetes* were enriched in non-continuous cropping roots and large soils, while *Opilidicota* was abundant in the rhizosphere and endophytic fungi of the continuous cropping group. The analysis of the LEFSE diagram showed that the specific genera of each root compartment were different. This proves that the colonization of certain fungi may be related to host plants and treatment groups (Zhu et al. 2020b). Some relatively stable symbiotic relationships may have formed between the three. It can be seen from the genus horizontal histogram that the key genus presents a gradient change from outside to inside in the three root compartments. Because of the specific selection of roots, the colonization of fungal communities on roots is more selective. Therefore, from the outside to the inside of the three root compartments, the structural characteristics of the fungal community showed a gradual change.

Microorganisms in plant root compartments are the comprehensive performance of the environment and plants, which can effectively reflect a certain trend of the changes of the two (Fierer 2017). In continuous cropping soil, the indexes of soil pH, catalase, urease, AK, and AN decreased, while the indexes of AP, EC, acid phosphatase, and sucrase increased. This is consistent with the changing trend of environmental factors in other plant continuous cropping studies (Lei et al. 2020; Wang et al. 2020; Zhu et al. 2020a). Continuous cropping will cause a drop in soil pH and changes in soil nutrients and enzyme activities (Arafat et al. 2019; Chen et al. 2020; Wu et al. 2016). The diversity and composition of the rhizosphere fungal community are more closely related to environmental factors than to bacteria. Environmental factors have a significant correlation with the function of the community and the taxonomic composition of fungi. Taken together, the analysis shows that environmental factors have a significant relevance with the fungal community and functional composition, but not with the bacterial community. Therefore, the response of the microbial community to environmental factors may affect the abundance and composition of plant-related microorganisms, thereby affecting the plant host.

Previous studies have shown that nitrogen and carbon metabolism will affect or be affected by the microbial communities structure (Liao et al. 2021). This study compared the effects of continuous cropping treatment and the non-continuous treatment on the function (FAPROTAX) of sugar beet root compartments bacterial community. According to the FAPROTAX, the continuous cropping treatment reduces the abundance of certain nitrogen metabolism and increases the abundance of carbon metabolism. According to reports, amino acid metabolism promotes the growth and activity of microorganisms by providing them with more carbon, nitrogen, and energy (Liang et al. 2020). Tax4Funt function prediction analysis can only predict the function of bacteria. The principle is that some microbial sequences are reclassified according to functional genes, and their absolute numbers are counted and

compared. Functional prediction analysis can be separated into three levels (level 1, level 2, and level 3). The first level is the largest and presents a gradual inclusion trend. Metabolism in the first class is dominant, while global and overview maps, carbohydrate metabolism, amino acid metabolism are dominant in the second level. In the third class, the metabolic pathways are dominant. However, there was no significant difference in metabolism in different groups ($p > 0.05$). This may be due to the large base of soil microbial species and numbers, where changes in the function of a few specific bacteria are not reflected in the overall data. There is also a specific group of terpenoids and polyketides that are metabolized in functional prediction and are expressed less in the continuous crop group than in the non-continuous group. This may cause the accumulation of terpenoids in continuous cropping root compartments. Terpenoids have been proved to be an important allelopathic substance that causes the obstacle of continuous cropping in plants (Zhang et al. 2014).

Conclusion

Our results focus on the response of microbial communities to continuous cropping obstacles. It reveals the structure and diversity of the microbial community in the beetroots compartment (bulk soil, rhizosphere, root) during continuous cropping and its relationship with soil nutrients. This will provide a theoretical basis for mitigating the obstacles to continuous crop sugar beet. At the same time, it has important theoretical significance to clarify the interaction mechanism between continuous cropping beetroot compartments and to understand the distribution of plant root compartments.

Declarations

Declaration of Competing Interest

The authors declare that they have no conflict of interests concerning the current research publication.

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Author Contributions

Rufei Cui and Gui Geng: Conceptualization, Methodology, Writing-Original draft preparation; Gang Wang, Yinzhuang Dong, and Tai Li: Data curation, Sample analysis; Yuguang Wang: Conceptualization, Resources, Supervision, Writing-Reviewing; Lihua Yu: Investigation, Sample analysis; Piergiorgio Stevanato: Methodology, Writing-Reviewing.

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Figures

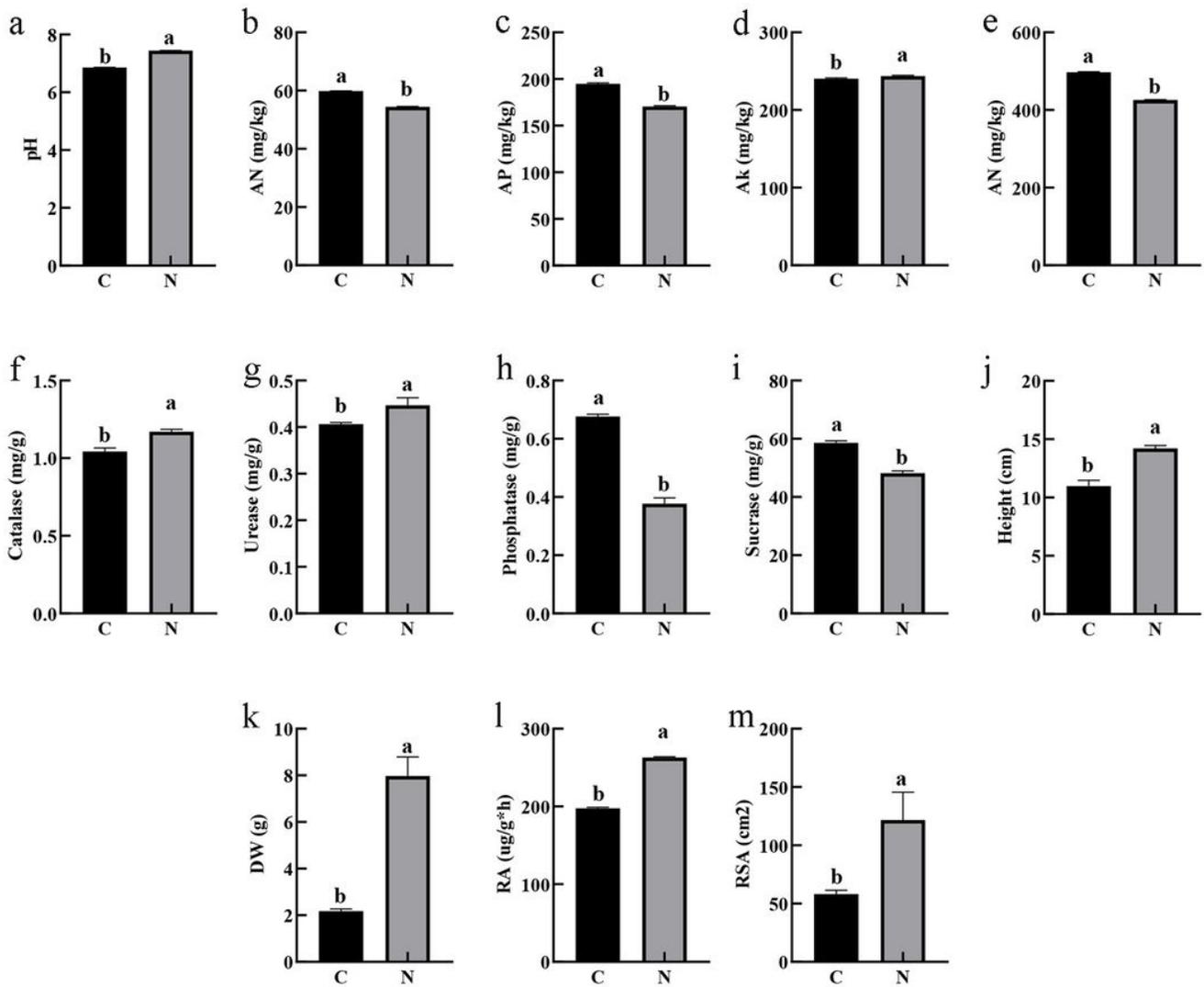


Figure 1

Soil physical and chemical properties, soil enzyme activity and plant growth indicators of different treatment groups. pH, AP, AK, EC represent available nitrogen, pH, available phosphorus, total potassium and available potassium. Height, DW, RA, RSA represent plant height, dry weight, root vigour, root surface area. C, continuous cropping bulk soil; N, non-continuous cropping bulk soil.

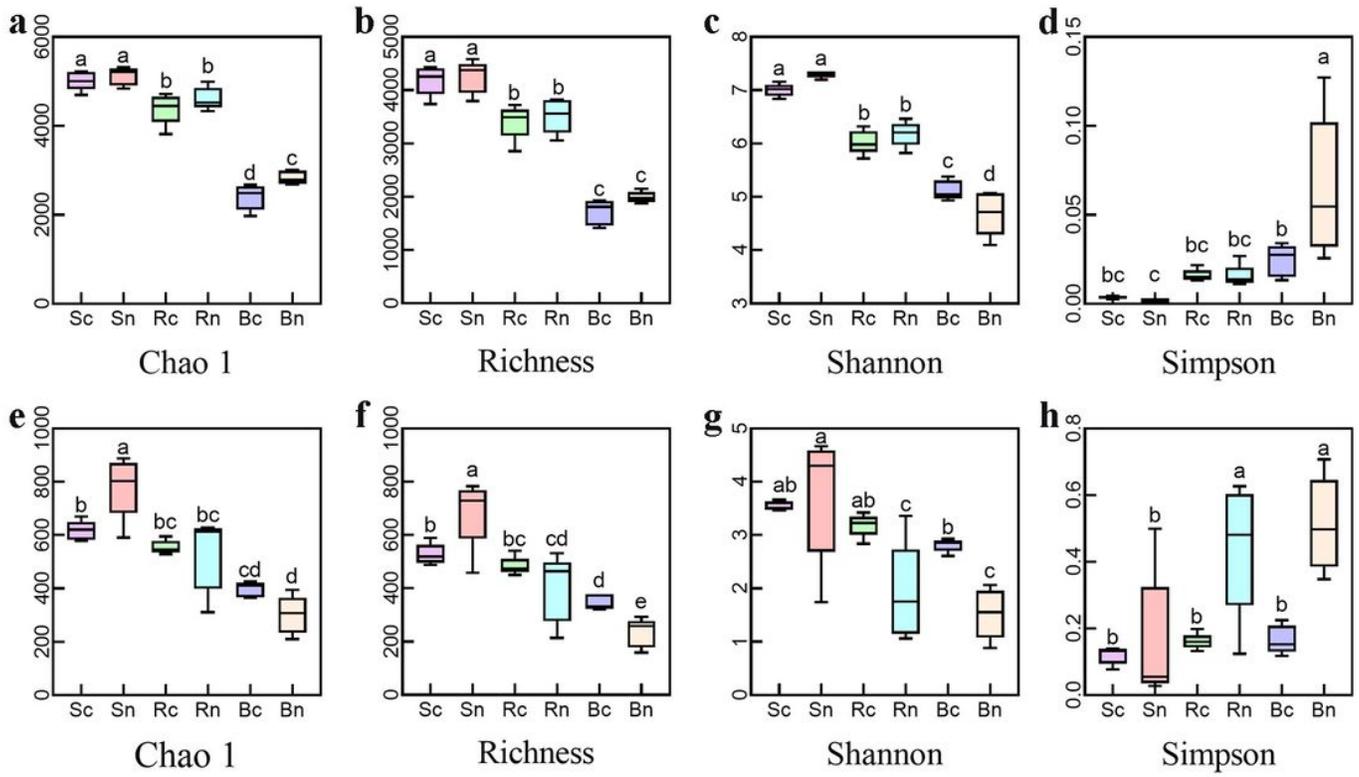


Figure 2

Alpha diversity of the bacterial and fungal communities. (a–d), representing alpha diversity of the bacterial community; (e–f), representing alpha diversity of the fungal community. Different letters ($P < 0.05$) were considered to have Significances on the top. Sc, continuous cropping bulk soil; Sn, non-continuous cropping bulk soil; Rc, continuous cropping rhizosphere soil; Rn, non-continuous cropping rhizosphere soil; Bc, continuous cropping sugar beet root; Bn, non-continuous cropping sugar beetroot.

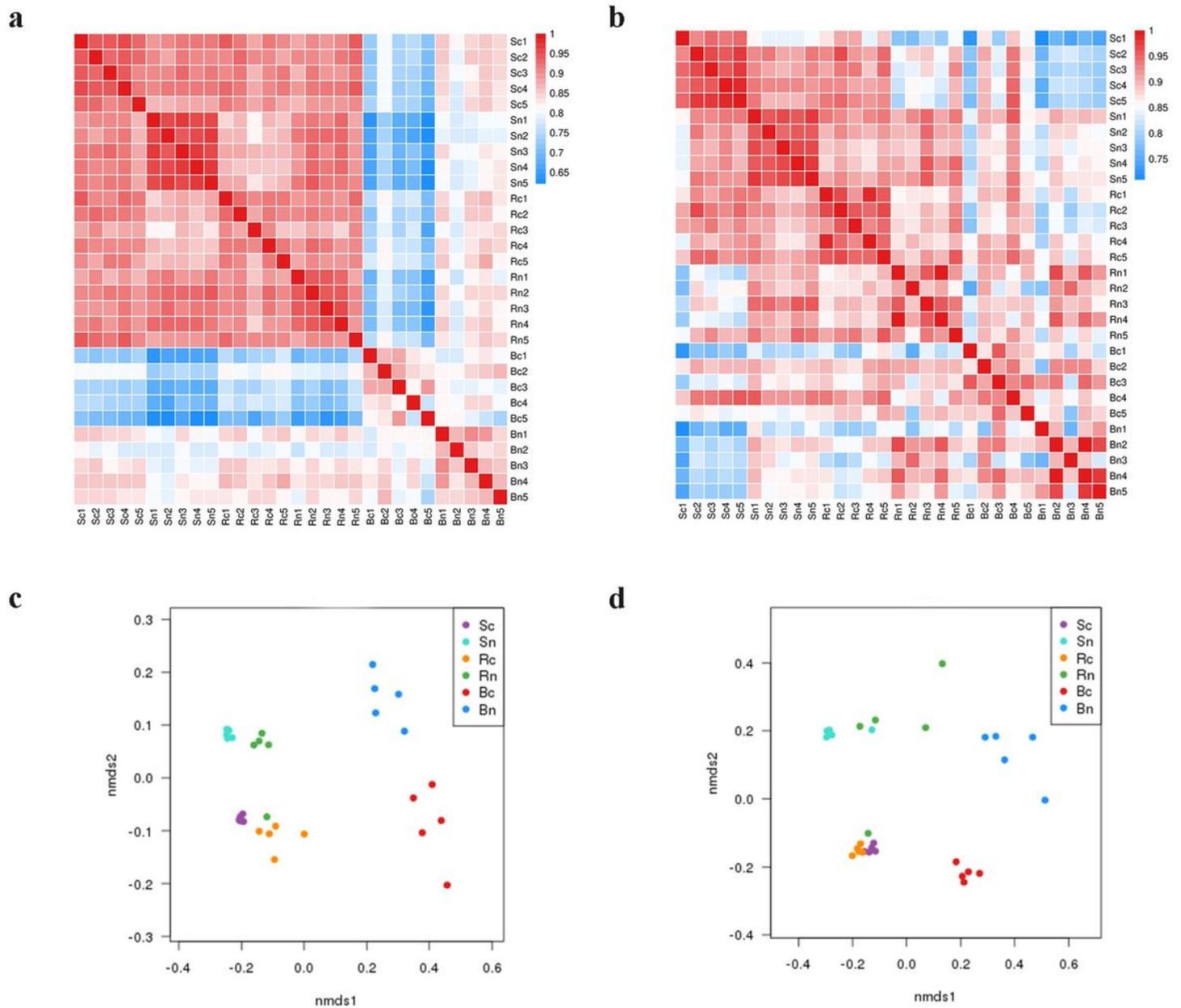


Figure 3

Spearman clustering heatmap and Unweighted UniFrac NMDS of the bacterial and fungal communities. (a, c), representing clustering heatmap and NMDS of the bacterial community; (b, d), representing clustering heatmap and NMDS of the fungal community. Sc, continuous cropping bulk soil; Sn, non-continuous cropping bulk soil; Rc, continuous cropping rhizosphere soil; Rn, non-continuous cropping rhizosphere soil; Bc, continuous cropping sugar beetroot; Bn, non-continuous cropping sugar beetroot.

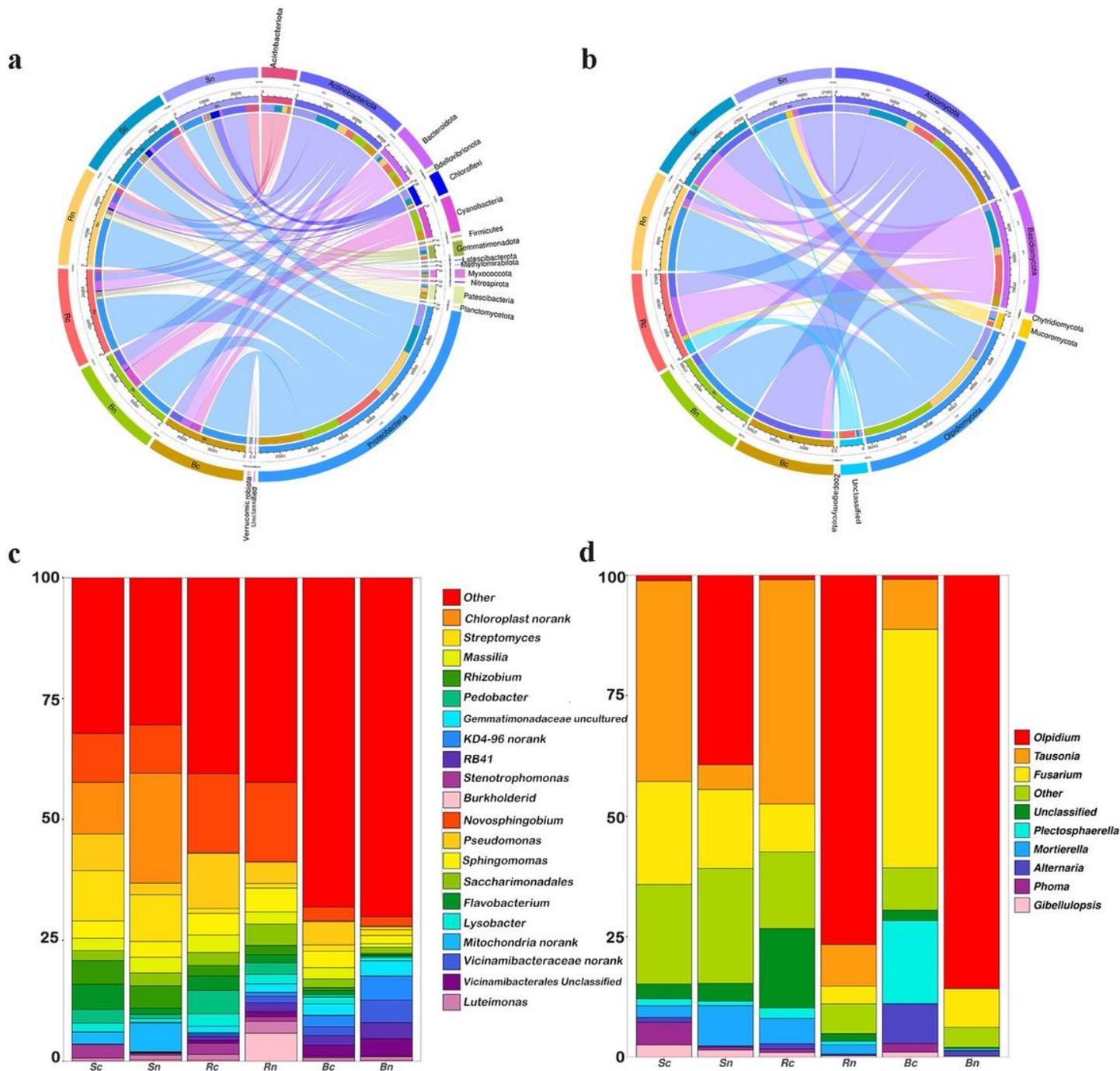


Figure 4

Taxonomic differences of the bacterial and fungal community. Distribution of the most abundant bacterial (a) and fungal (b) phyla. The area on the Outer left ring represents the contribution of each phylum in each sample. Distribution of the most abundant bacterial (c) and fungal (d) genera. Sc, continuous cropping bulk soil; Sn, non-continuous cropping bulk soil; Rc, continuous cropping rhizosphere soil; Rn, non-continuous cropping rhizosphere soil; Bc, continuous cropping sugar beetroot; Bn, non-continuous cropping sugar beetroot.

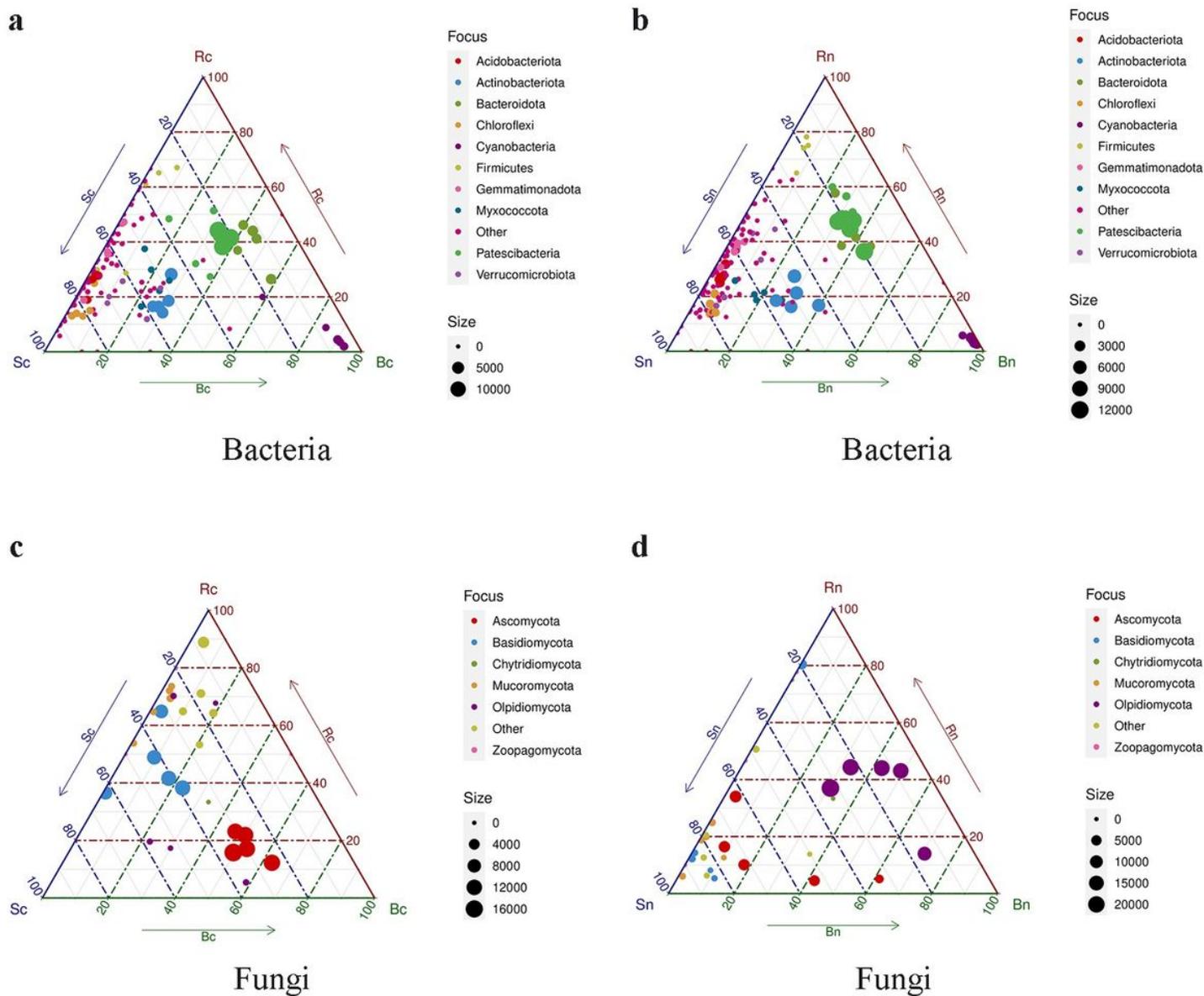


Figure 5

Ternary plots indicating compartment specificity of phyla from bacterial and fungal communities. Each color circle represents a phylum level. The dimension of each circle represents its weighted average. The position of each circle is decided by the contribution to the three compartments (bulk, rhizosphere, and endophytic) to the total weighted average. The dotted latticework of each triangle indicates 20% increments of contribution. (a, b), representing ternary plots of the bacterial community of two treatment groups; (c, d), representing ternary plots of the fungal community of two treatment groups. Sc, continuous cropping bulk soil; Sn, non-continuous cropping bulk soil; Rc, continuous cropping rhizosphere soil; Rn, non-continuous cropping rhizosphere soil; Bc, continuous cropping sugar beetroot; Bn, non-continuous cropping sugar beetroot.

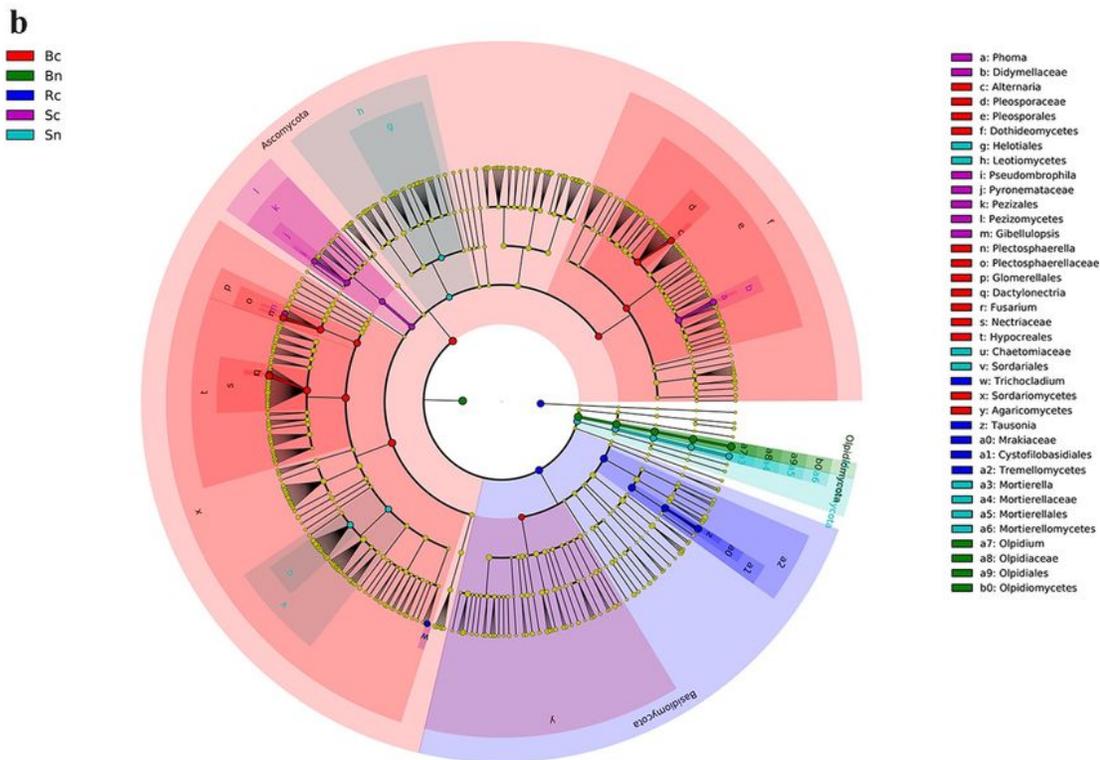
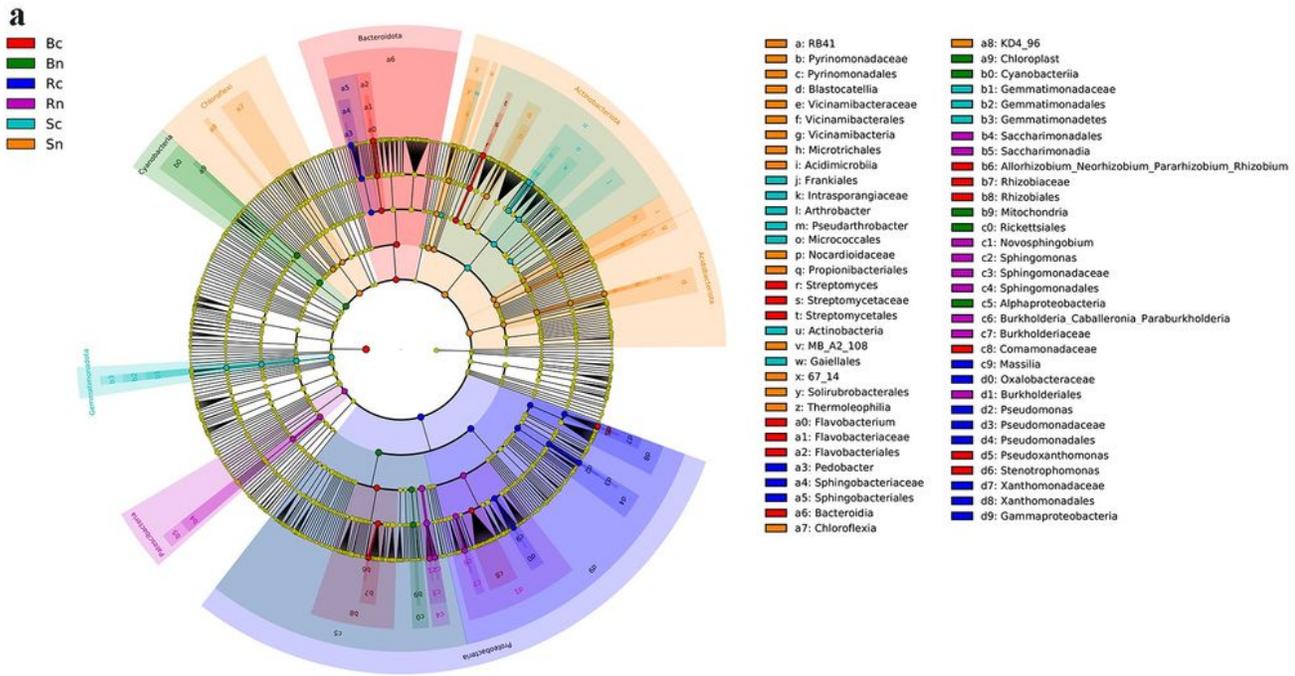


Figure 6

Cladogram obtained from LfSe analysis showing changes in different abundances of bacterial (a) and fungal (b) community at different taxonomic levels. The circles radiating from inside to outside represent the taxonomic level from phylum to genus. The different color nodes in the branches represent the microbial groups that play an important role in the color group. The species names represented by the English letters in the Fig are displayed in the legend on the right. Sc, continuous cropping bulk soil; Sn,

non-continuous cropping bulk soil; Rc, continuous cropping rhizosphere soil; Rn, non-continuous cropping rhizosphere soil; Bc, continuous cropping sugar beetroot; Bn, non-continuous cropping sugar beetroot.

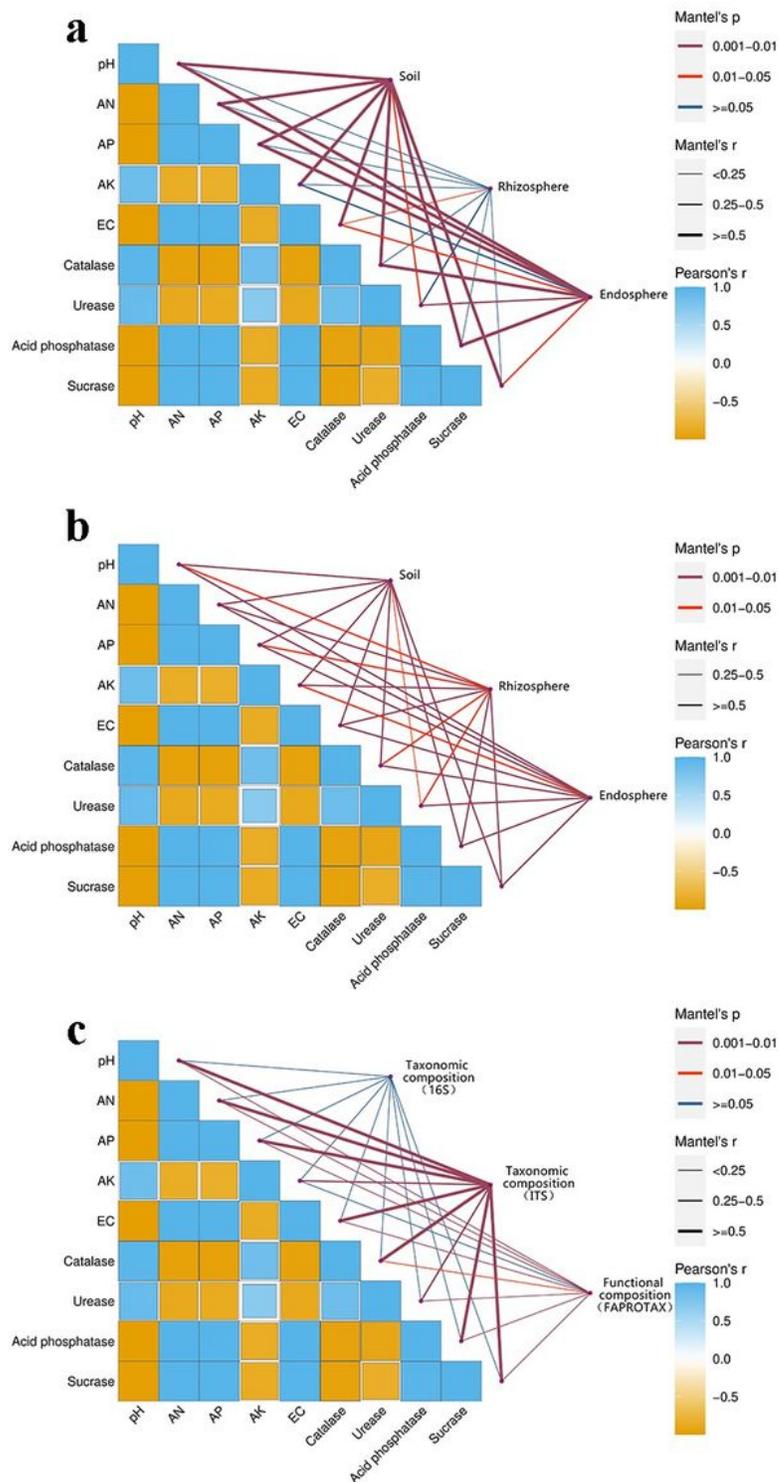


Figure 7

Environmental drivers of root compartments microbial community composition. Root compartments (based on bulk soil, rhizosphere, and endosphere) microbial community composition of bacteria (a) and

Heatmap of the predicted functional profile for the microbial communities at OUT level based on the Functional Annotation of Prokaryotic Taxa (FAPROTAX 1.1) database. Sc, continuous cropping bulk soil; Sn, non-continuous cropping bulk soil; Rc, continuous cropping rhizosphere soil; Rn, non-continuous cropping rhizosphere soil; Bc, continuous cropping sugar beetroot; Bn, non-continuous cropping sugar beetroot.

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