Microbial diversity in rhizosphere soil of soybean grass under different cultivation methods in an alpine region

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Abstract

Background

A large number of studies have shown that soybean grass with mixed seeding cultivation can significantly improve the yield and quality of forage grass compared with clean culture cultivation.This study explores the differences in the characteristics of the composition and diversity of the microbial community in the rhizosphere of soybean grasses between clean culture and mixed seeding methods in an alpine region. We used high-throughput sequencing technology to determine the microbial diversity and analytical methods to determine the physicochemical characteristics of plant rhizosphere soil of *Avena sativa* L. and *Vicia sativa* L.

Results

There were no significant differences in pH, total nitrogen, total phosphorus, and total potassium in the rhizosphere soil samples of soybean grasses under the clean culture and mixed seeding methods, while there were significant differences in the available nitrogen, available phosphorus, available potassium, and organic matter content ($P < 0.05$). The bacterial diversity of the rhizosphere soil of *Avena sativa* L. was the highest under the clean culture method, and the fungal diversity of the rhizosphere soil of *Vicia sativa* L. was the highest under the clean culture method. Furthermore, the microbial diversity of the rhizosphere was significantly different under the different cultivation methods ($P < 0.05$). The differences between the microbial species in the rhizosphere of the treated soil were at three class level. The abundance of Alphaproteobacteria and Actinobacteria in the rhizosphere of *Avena sativa* L. and *Vicia sativa* L. under the mixed seeding method was conspicuously higher than that of *Avena sativa* L. and *Vicia sativa* L. under the clean culture method, while the abundance of Gemmatimonadetes, Nitrospira, and Acidimicrobiia were significantly lower than that obtained under the clean culture method. Regarding fungal predominance, Mortierellomycetes was the most abundant (32.66%) under the mixed seeding method, while the abundance of Sordariomycetes and Leotiomycetes were significantly lower than that under clean culture. The distribution of bacterial and fungal community species in the rhizosphere differed significantly between the treatments. The Kyoto Encyclopedia of Genes and Genomes metabolism analysis showed that the metabolic pathways of functional genes in the soil microbial communities were similar.

Conclusions

Mixed sowing changed the diversity of plant rhizosphere microbial community structure and promoted plant yield.

Background

Forage is a necessity for the survival of animal husbandry and the fundamental driving force for ensuring the sustainable development of animal husbandry. The development of the forage industry aims to promote the development of forage cultivation and pursue high-yield cultivation. A change in cultivation methods is a new technique for the high-yield cultivation of forage. A large number of studies have shown that soybean grass with mixed seeding cultivation can significantly improve the yield and quality of forage grass compared with clean culture cultivation, and the development of mixed soybean grassland is a feasible method to increase forage yield and quality [1–8]. *Avena sativa* L. and *Vicia sativa* L. are fine forage grasses widely cultivated in alpine areas with strong resistance and of excellent quality [9–12]. They are widely cultivated in China and abroad to provide high-quality feed for domestic animals in mixed cultivation [13, 14].

Grassland soil microorganisms play an important role in the grassland ecosystem [15, 16]. There are a large number of microorganisms in the rhizosphere soil of plants. Due to the combined effect of microbial activities and plant metabolism, differences in soil characteristics, microbial community composition, nutrient content, and other aspects exist between the rhizosphere soil and non-rhizosphere soil of plants [17, 18]. The diversity of soil microorganisms is an important indicator that can directly reflect the stability and diversity of the plant ecosystem, and it can also visually demonstrate the differences in the function, communities, and species of soil microorganisms in different regions[19]. As an important indicator to describe the characteristics of the soil microbial community, the functional diversity of the soil microbial community can reflect the basic information of the soil [20]. The structure and diversity of the microbial community in rhizosphere soil are usually influenced by cultivation conditions, the planting environment, plant species, and other factors [21–23]. Compared with previously employed methods, such as traditional DNA sequencing, high-throughput sequencing technology can greatly reduce the research cost, save time, and yield a large amount of information with a higher level of accuracy and sensitivity [24–26]. At present, there have been many studies on different mixed-planting ratios and grass yield in seeding methods of soybean grass [27–29], but there are few studies of the rhizosphere microbial diversity of forage under different cultivation methods.

In this paper, we used the rhizosphere soils of *Avena sativa* L. and *Vicia sativa* L. under different cultivation methods in an alpine region as research objects to analyze the changes of microbial diversity using high-throughput sequencing so as to reveal the differences in microbial diversity of the rhizosphere soil of plants under different cultivation methods and to analyze structural and composition changes in the microbial community, thereby providing a basis for the study of microbial ecology of the rhizosphere soil of cultivated forages.

Results

Soilphysicochemical properties soil

The physicochemical properties of the soil samples are shown in Table 1. There was no significant difference in pH or in the total nitrogen, total phosphorus, or total potassium content of the soil samples

with the three different treatments. Overall, the pH value was 7.50–7.73, the total nitrogen content was 3.16–4.13 g•kg^{-1}, the total phosphorus content was low at 1.63–1.91 g•kg^{-1}, and the total potassium content was 22.01–22.92 g•kg^{-1}. The content of available nitrogen, available phosphorus, available potassium, and organic matter in the soil samples were significantly different among the three treatments ($P < 0.05$). The available nitrogen content was 210.11–275.17 mg•kg^{-1}, available phosphorus was 8.41–18.71 mg•kg^{-1}, and available potassium content was 67.11–12.18 mg•kg^{-1}. The available nitrogen, available potassium, and organic matter contents in the CYCH treatment soil were significantly higher than those in the other treatments ($P < 0.05$), and the contents of available phosphorus in the CYD treatment soil were significantly higher than those in the other treatments ($P < 0.05$).

Table 1
Soil nutrient content

Soil indicators	CWD	CYD	CYCH
pH	7.73 ± 0.45^{a}	7.50 ± 0.36^{a}	7.65 ± 0.38^{a}
Total nitrogen (g/kg)	3.16 ± 0.22^{a}	3.97 ± 0.27^{a}	4.13 ± 0.33^{a}
Total phosphorus (g/kg)	1.63 ± 0.06^{a}	1.91 ± 0.08^{a}	1.85 ± 0.05^{a}
Total potassium (g/kg)	22.92 ± 1.24^{a}	22.01 ± 1.18^{a}	22.01 ± 1.24^{a}
Available nitrogen (mg/kg)	210.11 ± 13.2^{c}	248.08 ± 17.14^{b}	275.17 ± 22.07^{a}
Available phosphorus (mg/kg)	11.24 ± 2.36^{b}	18.71 ± 3.57^{a}	8.41 ± 2.68^{c}
Available potassium (mg/kg)	94.07 ± 8.41^{b}	67.11 ± 7.65^{c}	120.18 ± 10.18^{a}
The organic matter (g/kg)	51.67 ± 4.07^{c}	69.35 ± 5.58^{b}	75.83 ± 6.47^{a}
Note: Different lowercase letters on the same line indicate significant differences ($P < 0.05$)			

Otu-level Analysis Of Soil Bacteria And Fungi

The results of the OTU statistical analysis are shown in Table 2. The 16S rDNA V3–V4 region of soil samples from all three treatments (CWD, CYD, and CYCH) was sequenced to obtain 48,988–50,302 effective tags. The tags lengths were highly concentrated, averaging 421 bp, 420 bp, and 417 bp for the CWD, CYD, and CYCH treatments, respectively. The sequence length of the 16S rDNA V3–V4 region in each treatment was roughly consistent. Following sequencing analysis, 32,031–42,291 effective tags were obtained from the ITS regions of the DNA obtained from the soil samples of the three treatments Generally, the sequence length was 230–280 bp, and the average length was 243, 230, and 280 bp, for the CWD, CYD, and CYCH treatments, respectively. Rarefaction curves (Fig. 1a and b) were prepared based

on the results. It can be seen from Fig. 1 that the OTU level of the sample increased rapidly with the increase of sequencing fragments, and remained stable after reaching the peak.

Table 2
Tag information and OTU statistical analysis of rhizosphere soil samples

Target	Sample	Tags	Effective tags	Average tags (bp)	Percentage of effective (%)	No. OTUs
16S rDNA	CWD	67249 ± 243	48988 ± 241	421 ± 15	61.12 ± 1.24	1162 ± 1.76
	CYD	67594 ± 326	50302 ± 276	420 ± 17	63.06 ± 1.33	1209 ± 1.58
	CYCH	67396 ± 358	49918 ± 318	417 ± 12	62.75 ± 1.27	1127 ± 1.29
ITS	CWD	74762 ± 572	36891 ± 424	243 ± 18	46.31 ± 1.24	261 ± 2.65
	CYD	74496 ± 427	42291 ± 267	230 ± 14	53.17 ± 1.36	260 ± 1.87
	CYCH	74611 ± 410	32031 ± 211	280 ± 13	40.06 ± 1.18	211 ± 1.87

Analysis Of Soil Microbial Species Composition

The richness of microbial species composition was > 0.1% higher in the three soil treatment samples (Fig. 2). We detected many phyla and classes of bacteria and fungi, and most of them contained very few species. For the convenience of observation, only the top ten abundant fungi and bacteria are shown in Fig. 2 and the remaining have been grouped as Others. The Unclassified and Unknown groups represent species that were not taxonomically annotated. The ordinate (*y*-axis) shows the relative abundance. Figure 2a and b, respectively, show the top 10 classes of bacteria and fungi, respectively, according to their relative abundance level.

For all three treatments, the soil bacteria were mainly distributed in the following 10 classes: Alphaproteobacteria, Betaproteobacteria, Blastocatellia, Acidobacteria subgroup 6, Gemmatimonadetes, Nitrospira, Acidimicrobiia, Actinobacteria, Gammaproteobacteria, and Holophagae(Fig. 2a). Alphaproteobacteriawas the most abundant, with a relative abundance of 17.83%, 24.24%, and 18.67%, for the CWD, CYCH, and CYD treatments, respectively. For the CYCH treatment, the abundance of Alphaproteobacteria and Actinobacteria were significantly higher than for the CWD and CYD treatment. For the CYCH treatment, the abundance of Gemmatimonadetes, Nitrospira, and Acidimicrobiia was significantly lower than that of the CWD and CYD treatment. With the exception of Actinobacteria, the

relative abundance of the main bacterial species in the CWD and CYD soils was relatively close and it is quite different from that in CYCH soil.

As can be seen in Fig. 2b, for all three treatments, the soil fungi were mainly distributed in the following 9 groups: Mortierellomycetes, Sordariomycetes,Leotiomycetes, Dothideomycetes, Tremellomycetes, Agaricomycetes, Archaeorhizomycetes, Eurotiomycetes, Spizellomycetes, or Unclassified, of which Mortierellomycetes was the most abundant the CYCH treatment soil (32.66%). Compared with the CWD and CYD treatment soil, the abundance of Sordariomycetes and Leotiomycetes in the CYCH treatment soil was significantly decreased, while the distribution of fungal communities was significantly different between the soil treatments.

Analysis Of Soil Microbial Diversity

Alpha diversity analysis

As can be seen from the alpha diversity index results (Table 3), all three soil treatment samples have a high bacterial and fungal community diversity. The order of abundance-based coverage estimator (ACE) and Chao1 indices of bacterial alpha diversity in the three soil treatment samples were CYD > CYCH > CWD, indicating the highest abundance of bacterial species in the CYD treatment soil. The Simpson index showed CYCH > CWD > CYD. The Shannon index showed CYD > CYCH > CWD, indicating the richest diversity of bacterial microorganisms in the CYD treatment soil. The ACE and Chao1 indices of fungal alpha diversity in the three soil treatment samples were CWD > CYD > CYCH, showing that the fungal species abundance was highest in HNY soil. The Simpson index showed that CYCH > CYD > CWD. The Shannon index was ranked as CWD > CYD > CYCH. Thus fungal microbial diversity of the CWD treatment soil was the most abundant among the three treatments.

Table 3
Diversity index of soil microbial community in rhizosphere soil

Diversity indexes		CWD	CYD	CYCH
Bacterial	ACE	1244.7398 ± 5.42	1276.6826 ± 5.22	1247.6747 ± 4.89
	Chao1	1259.2143 ± 3.41	1286.5981 ± 3.24	1285.6355 ± 2.42
	Simpson	0.0068 ± 0.32	0.0063 ± 0.14	0.0086 ± 0.16
	Shannon	5.9163 ± 1.08	6.044 ± 1.23	5.9639 ± 1.18
Fungal	ACE	274.0142 ± 13.36	269.1001 ± 10.68	218.8791 ± 12.21
	Chao1	280.0909 ± 6.48	270.0 ± 3.79	217.6667 ± 5.66
	Simpson	0.029 ± 0.05	0.0298 ± 0.04	0.0526 ± 0.02
	Shannon	4.2945 ± 1.15	4.2487 ± 1.05	3.5755 ± 1.13
Note: Different lowercase letters on the same line indicate significant differences ($P < 0.05$)				

Analysis Of Beta Diversity

Heatmaps represent samples through interspecies distance relationships to obtain the sequence relationship between samples, which are drawn by means of matrix language tool software. By analyzing the heatmap, the difference between the two samples can be intuitively reflected by the range of color transformation. Figure 3 shows the heatmap of the three soil treatment samples. The bacterial flora (Fig. 3a) and fungal flora (Fig. 3b) in the CWD and CYD treatment soil had a high level of similarity.

The beta microbial diversity analysis was conducted to obtain a distance matrix. Then, the UPGMA function of R software was used for hierarchical clustering comparison of samples, and the similarity relationship of the species composition in each sample was described. The closer the samples were, the shorter the branch length was, indicating that the species composition of the two samples was more similar. Fig. 4 shows that the OTU level of bacteria and fungi in the CWD treatment soil samples had a relatively high consistency, while the similarity of bacteria and fungi in the CYCH treatment soil sample was relatively low.

Functional gene prediction

PICRUSt software (http://picrust.github.io/picrust/) was used to compare the species composition information obtained from the 16S sequencing tags to infer the functional gene composition of the samples and then analyze the functional differences between the different soil treatments. According to the of Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolism histogram (Fig. 5), the functional

genes of the soil microbial community for each treatment were basically similar in their metabolic pathways.

Discussion

Previous studies by many scholars have confirmed that the abundance and flora composition of soil microbial populations are closely related to pH, organic matter content, water content, the content of various nutrients, soil particle size, and other physical and chemical properties of soil [37–42]. In this study, there were no significant differences in pH, total nitrogen, total phosphorus, and total potassium in the three plant rhizosphere soil treatment samples of *Avena sativa* L. and *Vicia sativa* L. under clean culture and mixed seeding cultivation, but there were significant differences in available nitrogen, available phosphorus, available potassium, and organic matter content ($P < 0.05$). The content of available nitrogen, available potassium, and organic matter of soil of *Avena sativa* L. and *Vicia sativa* L. in the mixed seeding treatment were significantly higher than those in the other treatments ($P < 0.05$), and the content of available phosphorus of *Avena sativa* L. in the clean culture treatment was significantly higher than in the other treatments ($P < 0.05$). The relationship between plant rhizosphere microorganisms and soil nutrient content requires further study.

Rhizosphere microorganisms play an important role in connecting the soil and plant microecosystems. Plant root secretions can provide energy for the life activities of microorganisms, and microorganisms can affect plants by decomposing organic matter and transforming nutrient substances or indirectly affect plant growth and the dynamics of non-rhizosphere microorganisms via material cycling and energy flow [43, 44]. Yu et al. [45] studied the diversity of rhizosphere and rhizome microorganisms cultivated by *Impatiensbalsamina* in Hainan, China, and the seasonal variation of its community at different altitudes. The results showed that the number of rhizospheric fungi and bacterial OTUs, Shannon–Weiner index, and richness index varied greatly with altitude and with the wet and dry seasons. Lijun et al. [46] studied the microbial diversity in rhizosphere soil of *Davidiainvolucrata* in different regions. They found that the microbial communities in the rhizosphere soil of *Davidiainvolucrata* in three regions all had high biodiversity, but there were differences in species composition and distribution. Yating et al. [47] studied the rhizosphere microbial community diversity of maize in Yunnan, China, and they concluded that altitude and variety type were important factors influencing the rhizosphere microbial community diversity of maize. In our study, the microbial diversity in rhizosphere soil of soybean grass was studied under different cultivation methods. The results showed that the bacterial microorganism diversity in rhizosphere soil was the highest when *Avena sativa* L. was under clean culture cultivation, while the bacterial microorganism diversity in rhizosphere soil of *Vicia sativa* L. under clean culture cultivation was the lowest. The fungal microorganism diversity of rhizosphere soil of *Vicia sativa* L. under clean culture cultivation was the highest, and the fungal microorganism diversity of rhizosphere soil under mixed seeding of *Avena sativa* L. and *Vicia sativa* L. was the lowest. The diversity of rhizosphere microorganisms was significantly different under different cultivation methods, which was consistent with the results of other researchers. In similar environments, the structure of microbial communities is determined by the plant types, and the structure composition of soil microbial communities varies

between different vegetation types [48]. The change in soil microbial diversity depends on soil water content, pH, aeration, nitrogen content, and organic carbon affected by vegetation. Based on the characteristic analysis of rhizosphere exudates and plant litters, the main factors affecting soil microbial diversity may be vegetation type and quantity [49]. In this study, the species structure of the plant rhizosphere microbial community under the three treatments was at class level, and the abundance of Alphaproteobacteria and Actinobacteria was significantly higher in the mixed sowing treatment of *Avena sativa* L. and *Vicia sativa* L. than that in clean culture treatment of both *Vicia sativa* L. and *Avena sativa* L. The abundance of Gemmatimonadetes, Nitrospira, and Acidimicrobiia was significantly lower than that of soybean grass under clean culture. The Mortierellomycetes of fungi were most abundant under mixed seeding treatment (32.66%), while the abundance of Sordariomycetes and Leotiomycetes was significantly lower than that of soybean grass under clean culture, while the species distribution of bacteria and fungi communities in soil was significantly different under the different various treatments. The KEGG metabolic analysis showed that the functional genes of soil microbial communities were similar in their metabolic pathways. The structure and diversity of the microbial community in the rhizosphere of plants changes greatly under different cultivation modes, and the influence of plants, soil, and the environment required further study.

Conclusions

In this study, there were no significant differences in pH, total nitrogen, total phosphorus, and total potassium in the three plant rhizosphere soil treatment samples of *Avena sativa* L. and *Vicia sativa* L. under clean culture and mixed seeding cultivation, but there were significant differences in available nitrogen, available phosphorus, available potassium, and organic matter content ($P < 0.05$). The contents of available nitrogen, available potassium, and organic matter of soil of *Avena sativa* L. and *Vicia sativa* L. under mixed seeding treatment were significantly higher than those under the other treatments ($P < 0.05$), and the available phosphorus content in the soil of *Avena sativa* L. under clean culture treatment was significantly higher than those of other treatments ($P < 0.05$).

We investigated the microbial diversity in rhizosphere soil of soybean grass under different cultivation methods. The results showed that the bacterial microbial diversity in rhizosphere soil of *Avena sativa* L. was the highest under clean culture, while the bacterial microbial diversity in rhizosphere soil of *Vicia sativa* L. was the lowest under clean culture. The fungi microbial diversity in rhizosphere soil of *Vicia sativa* L. was the highest under clean culture and the lowest under the mixed cultivation of *Avena sativa*L. and *Vicia sativa* L. There were significant differences in rhizosphere microbial diversity under different cultivation methods.

In this study, the species structure of the rhizosphere microbial community was at the class level under the three treatments. The abundance of Alphaproteobacteria and Actinobacteria under the mixed cultivation of *Avena sativa* L. and *Vicia sativa* L. was significantly higher than that of *Avena sativa* L. and *Vicia sativa* L. under clean culture cultivation, and the abundance of Gemmatimonadetes, Nitrospira, and Acidimicrobiia was significantly lower than that of soybean grass under clean culture. The

Mortierellomycetes class of fungi was the most abundant under mixed seeding treatment (32.66%), while the abundance of Sordariomycetesand Leotiomycetes was significantly lower than that of soybean grass under clean culture. The species distribution of the bacteria and fungi community in soil microorganisms was significantly different between the soil treatments. KEGG metabolic analysis showed that the functional genes of soil microbial communities were similar in their metabolic pathways.

Methods

Overview of the experimental area

The experiment was conducted at Sanjiangyuan Field Ecological Observation Station of Qinghai University (33°24'30" N, 97°18'00" E), which is located in Zhenqin Town, Chengduo County, Yushu Prefecture, Qinghai province, China. The experimental area is situated at an altitude is 4,270 m and has a typical plateau continental climate, with an annual average temperature of − 5.6 °C to 3.8 °C and an annual average precipitation of 562.2 mm, mainly distributed from June to September. Its alpine meadow soil has a rich humus content but poor soil fertility due to poor decomposition. The soil pH is 7.32, organic matter content is 58 g $\cdot kg^{-1}$, and available potassium, phosphorus, and nitrogen is 76.5, 7.0, and 14.0 mg $\cdot kg^{-1}$, respectively, of which ammonia nitrogen is 5.1 mg $\cdot kg^{-1}$ and nitrate nitrogen is 8.9 mg $\cdot kg^{-1}$. The area is irrigated during droughts.

Experimental Materials And Design

This experiment was designed as a single-factor randomized block study. The experimental materials were *Avena sativa* L. and *Vicia sativa* L., the seeds were provided by the Key Laboratory of Superior Forage Germplasm in the Qinghai-Tibetan Plateau. We set up three treatments: *Vicia sativa* L. with clean culture cultivation (CWD), *Avena sativa* L. with clean culture cultivation (CYD), and a mixture of *Avena sativa* L. and *Vicia sativa* L. in equal proportions (CYCH). There were three plots of 4×5 m^2 each. The plots were drilled, and the rows were spaced at intervals of 30 cm. Diammonium phosphate (30 kg/mu) was applied prior to sowing. The amount of seed used in the plot was calculated according to the plot area. The amount of *Avena sativa* L. under clean culture cultivation was 15 kg/mu, that of *Vicia sativa* L. under clean culture cultivation was 5 kg/mu, and the mixed sowing amount is was 7.5 kg/mu of *Avena sativa* L. plus 2.5 kg/mu of *Vicia sativa* L.

Sample Collection

The experiment initiated in May 2019 and the experimenter visited the plot in August 2019. Using a five-point sampling method, root samples ≤ 20 cm long were collected in triplicate from *Avena sativa* L. and *Vicia sativa* L.. The large soil particles were shaken off using the shake-down method [30], and then a brush was used to brush off excess soil surrounding the roots, leaving a layer of 0–5 mm of soil, which

was considered the rhizosphere soil. The two species were evenly mixed, placed into sterile Ziplock bags, stored at a low temperature, and brought back to the laboratory as quickly as possible. The collected soil samples for each treatment were divided into two parts, one to determine the nutrient content and the other to determine the microbial diversity index of the soil.

Determination Of Soil Physicochemical Properties

The indices of the physicochemical properties of the soil samples were determined by conventional analysis methods [31]. Soil pH was determined using a pH potentiometer method. Organic matter content was determined by high-temperature potassium dichromate oxidation capacity, available nitrogen content by a potassium chloride extraction-spectrophotometric method, available phosphorus content by sodium bicarbonate extraction and molybdenum-antimony colorimetry, and available potassium content by an ammonium acetate extraction-flame photometer method. Total nitrogen content was determined by Nessler colorimetry, total phosphorus content by molybdenum-antimony colorimetry, and total potassium content by a flame photometer method.

Total Dna Extraction From Soil Microorganisms

The total genomic DNA was extracted from the soil microorganisms using an OMEGA soil DNA extraction kit. Each sample was mixed before processing in triplicate to reduce the chances of missing DNA from organisms that were sparsely distributed. Following extraction, polymerase chain reaction (PCR) amplification of the V3–V4 region of the bacterial 16S rRNA gene and the fungal internal transcribed spacer (ITS)1 region was performed using universal primers (bacterial V3–V4 region: 341F, 5′-CCTACGGGNGGCWGCAG-3′ and 805R, 5′-GACTACHVGGGTATCTAATCC-3′ and fungal ITS1 region: ITS1F, 5′-CTTGGTCATTTAGAGGAAGTAA-3′ and ITS2R, GCTGCGTTCTTCATCGATGC-3′). The PCR amplification conditions were performed with reference to the method used by Zhao et al. [32]. The amplified products were purified and quantified, and a sequencing library was constructed, which was sequenced by Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China) on an Illumina HiSeq PE250 high-throughput sequencing platform.

Bioinformatics Analysis

After cutting the barcode and primer sequences from raw data, the reads of each sample were spliced to obtain the raw tags. The raw tags were inspected and the chimeras were removed to obtain effective tags. We used Qiime software (http://qiime.org/) to perform operational taxonomic unit (OTU) clustering when the sequence similarity was > 97%. We used mothur software (https://www.mothur.org/) [33], the Silva ribosomal RNA sequence database (https://www.arb-silva.de/), and the UNITE fungal DNA database (https://unite.ut.ee/) [34]to annotate species (threshold value, 0.8–1.0) to determine the classification information of the sequence and obtain taxa at all levels of phylum, class, order, family, and

genus. The PyNAST tool (https://omictools.com/pynast-tool) [35] was used for multi-sequence comparison with data information in the Greengenes database (https://greengenes.secondgenome.com/) [36]. Finally, the tags were standardized, and Qiime l.7.0 software was used to calculate the alpha diversity index and perform the beta diversity analysis. We plotted the dilution curve using the vegan package of R 2.15.3 software (https://www.r-project.org/). The R language tool was used to draw the heatmap, and the unweighted pair-group method with arithmetic mean (UPGMA) was used to draw the phylogenetic tree by hierarchical clustering.

Data analysis

All data were processed and analyzed using SPSS 21.0 software (IBM Corp. in Armonk, NY). One-way analysis of variance and Duncan's new multiple range test were used to analyze the significant differences.A probability value (*P*) of < 0.05 was considered statistically signifificant.

Abbreviations

ACE: abundance-based coverage estimator; CWD:Vicia sativa L. with clean culture cultivation; CYCH:a mixture of Avena sativa L. and Vicia sativa L. in equal proportions; CYD:Avena sativa L. with clean culture cultivation; ITS:internal transcribed spacer; KEGG:Kyoto Encyclopedia of Genes and Genomes; OTU:operational taxonomic unit; PCR:polymerase chain reaction; UPGMA:unweighted pair-group method with arithmetic mean

Declarations

Anailability of data and materials

All data generated of analysed of this study are described in this paper.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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Authors' Contributions

YZ and ZZ designed the experiments. BS, RD and YW conducted the experiments. YZ and BS performed the experiments and wrote the main manuscript text. RD and YW prepared the Figs. YZ, ZZ, BS, RD and YW analyzed the data and reviewed the manuscript. All authors have read the manuscript and approved its final version.

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Figures

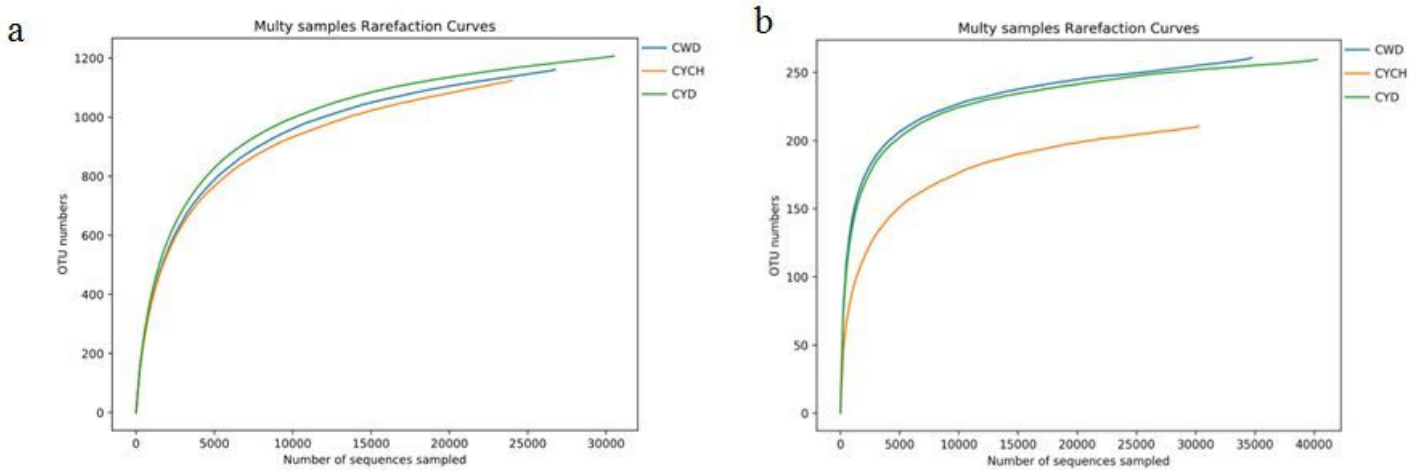

Figure 1

Sequence dilution curves of bacteria and fungi in the rhizosphere. a.Number of sequencing(Bacteria); b. Number of sequencing (Fungus)

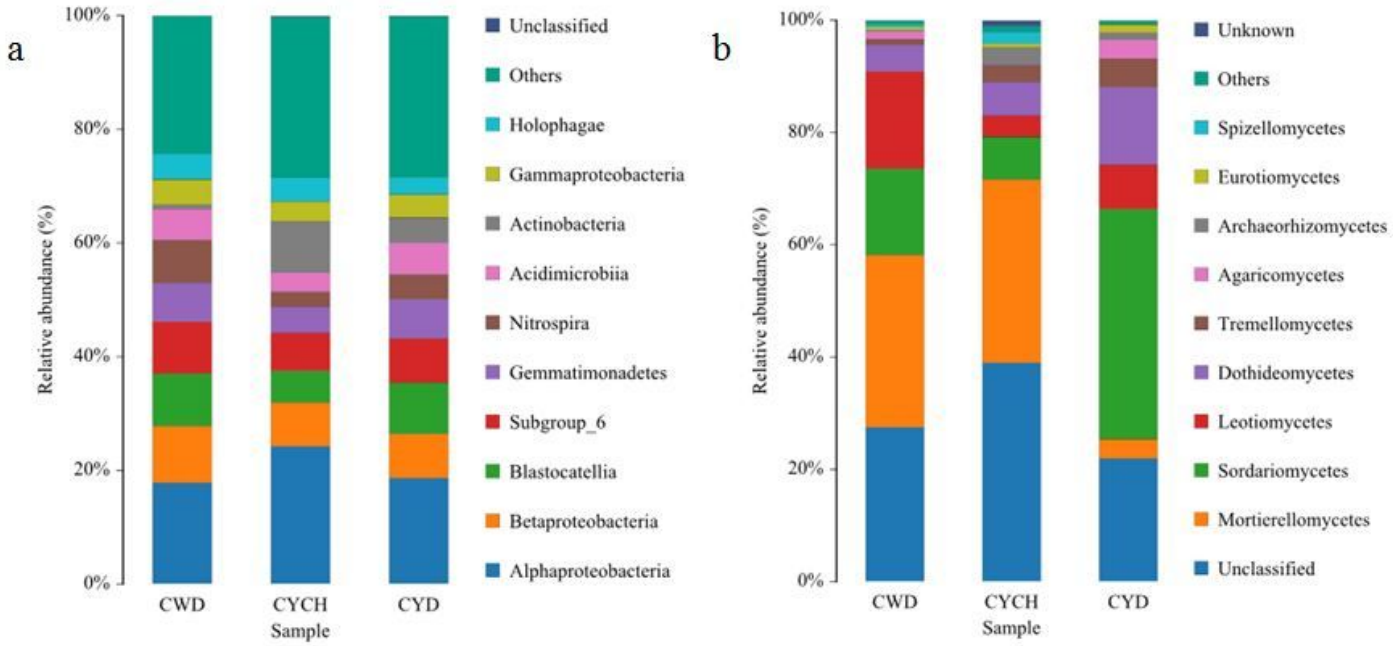

Figure 2

Distribution of bacteria and fungi in rhizosphere soil samples. a. Community distribution of Bacteria; b. Community distribution of Fungus

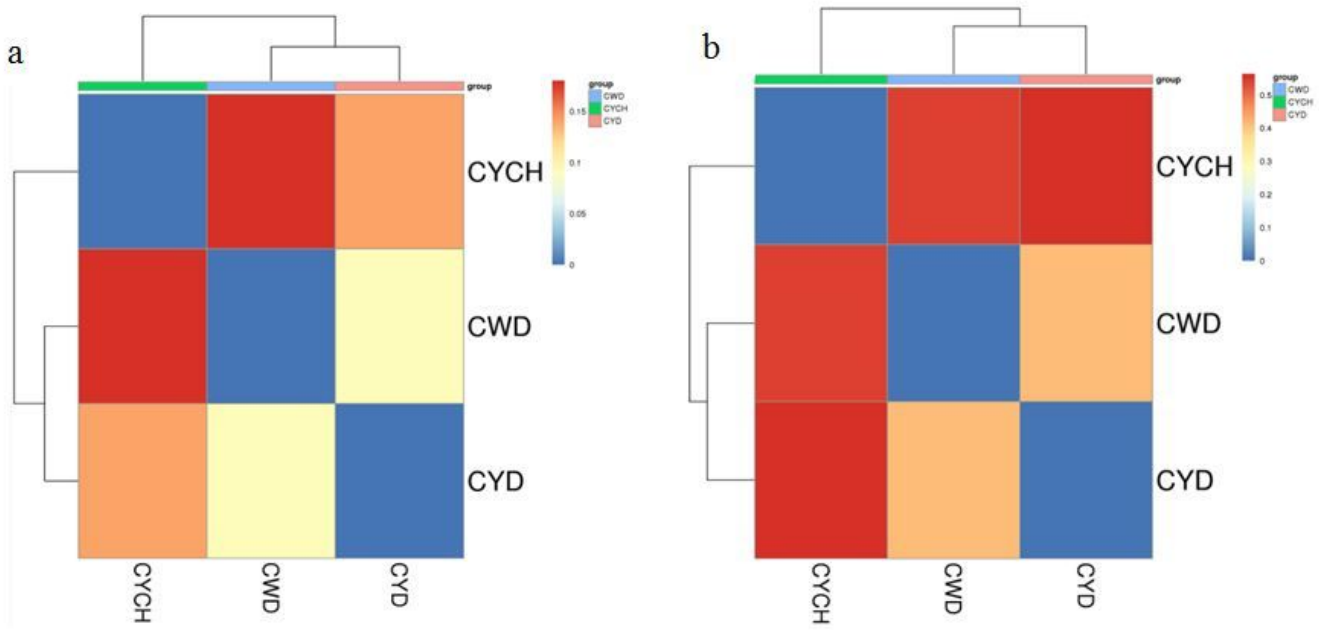

Figure 3

Heatmap diagram of weighted UniFrac for rhizosphere soil samples. a.Bacteria community; b. Fungus community

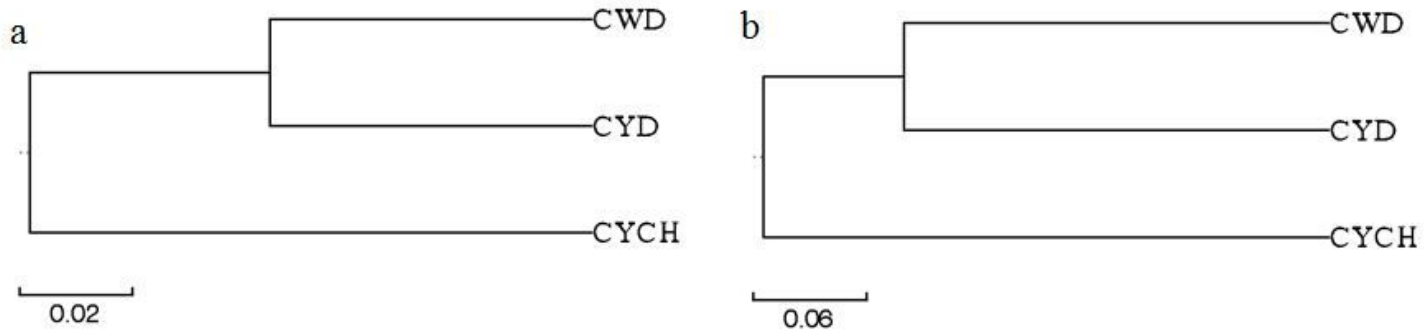

Figure 4

Dendrogram of rhizosphere soil samples based on out. a.Bacteria community; b. Fungus community

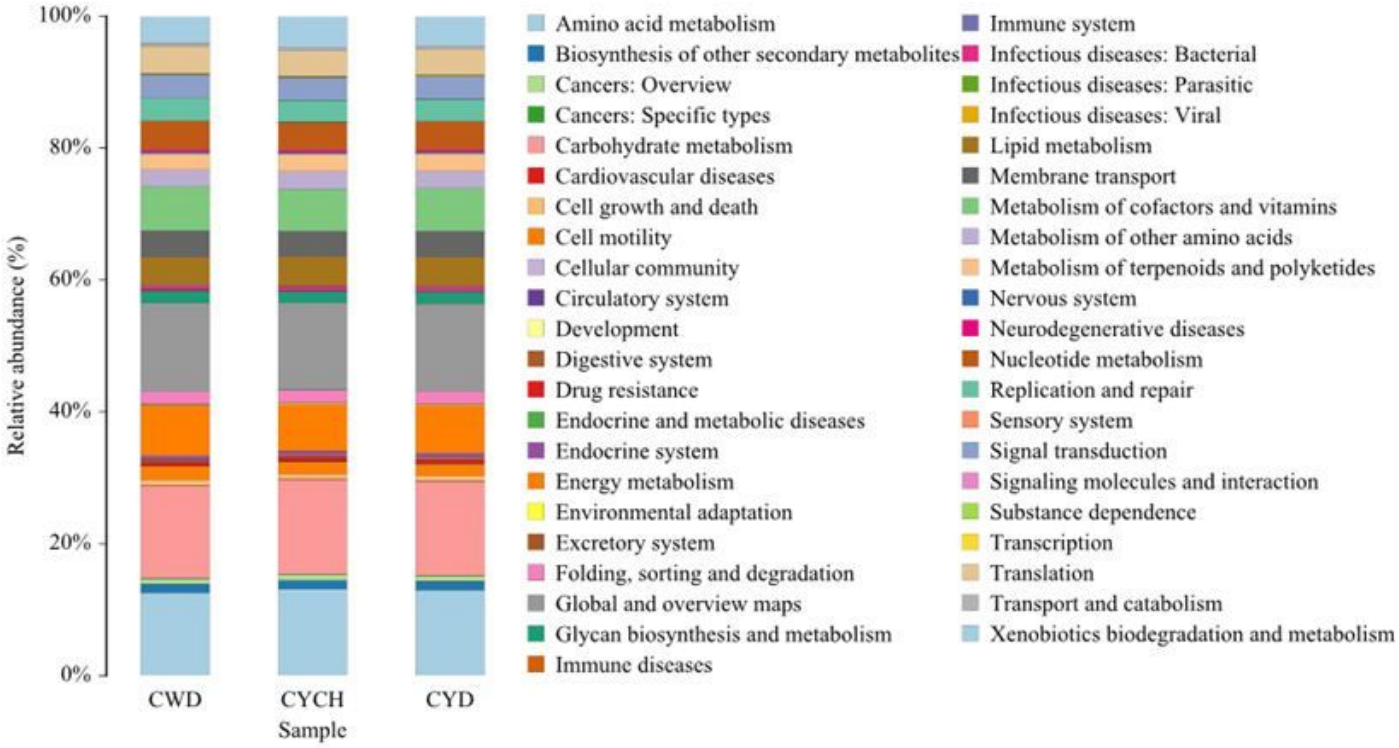

Figure 5

Histogram of KEGG metabolism of soil samples