



Rhizosphere soil microbial communities under foxtail millet continuous and rotational cropping systems and their feedback effects on foxtail millet downy mildew suppression

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Abstract

Foxtail millet downy mildew caused by obligate parasitic Oomycetes *Sclerospora graminicola* (Sac.) Schrot greatly reduces the foxtail millet yield. The purpose of this study was to find the specific bacterial and fungal populations that might be involved in the suppression of foxtail millet downy mildew. Bacterial and fungal communities in foxtail millet rhizosphere soils under two different cropping systems were compared, with high-throughput Illumina sequencing technology. Compared with foxtail millet continuous cropping system, the foxtail millet–soybean–potato (Si–Gm–St) rotational cropping system showed the lower foxtail millet disease incidence (3.00%) and higher yield (3892.00 kg ha⁻¹). The Bacteria/fungi value of foxtail millet rhizosphere soil in Si–Gm–St rotational cropping system was higher than that in foxtail millet continuous cropping system. The enzyme activities of phosphatase, urease and sucrase showed the same trend. Lower abundance of pathogenic microorganisms and higher abundance of plant growth-promoting microorganisms were observed under the Si–Gm–St rotational cropping system. There was a significant negative correlation between the relative abundances of *Nocardioidea*, *Devosia*, *Neorhizobium* and *Pseudomonas* and foxtail millet downy mildew incidence, but an extremely significant positive correlation between the relative abundances of *Fusarium*, *Cylindrocarpum*, *Ilyonectria*, *Thermoascus*, *Myrothecium* and the disease incidence ($P < 0.01$). Different foxtail millet cropping systems were found to occupy different rhizosphere bacterial and fungal communities, and high bacterial diversity and low fungal diversity might be associated with the effective mitigation of downy mildew.

Keywords 16SrDNA · ITS · Chemical property · Enzyme activity · Foxtail millet downy mildew · Illumina sequencing

Introduction

Foxtail millet (*Setaria italica* L.) originating in China, is mainly grown in arid and semi-arid regions of the world (Xu et al. 2018). Downy mildew is the most destructive

disease in foxtail millet production, caused by *Sclerospora graminicola* (Sac.) Schrot, and classified as Oomycetes (Michie et al. 2017). The pathogen oospores of foxtail millet downy mildew can be easily blown into the field by the wind after they are dry at the hair of the infected plant and on the head of the hedgehog, and then overwinter in the soil. After the seeds are sown in the next year, it will infect the plant as the foxtail millet germinates and grows. Therefore, under the millet continuous cropping system, the amount of pathogenic oospore in the soil will multiply year by year, and the downy mildew incidence will correspondingly increase. However, few reports on the prevention of this destructive disease.

Microbes in plant rhizosphere soils form an interactional mechanism that greatly improves soil quality and plant adaptability (Berendsen et al. 2012). For example, soil microbial communities secreting antibacterial properties can protect plants from soil-borne pathogens (Lugtenberg

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and Kamilova 2009). In addition, specific microorganisms can enhance crop yield by improving soil nutrient availability, degrading soil toxic compounds, and inhibiting the reproduction of soil pathogens (Bais et al. 2006; Wu et al. 2017). In recent years, the relationship between plant disease resistance and rhizosphere microbial diversity has been extensively studied in plant-pathogen interaction system, such as *Candidatus Liberibacter asiaticus* of Citrus (Wu et al. 2014), *Arbuscular mycorrhizal* of maize-wheat rotational system (Hu et al. 2015), *Ustilago crameri* Korn of foxtail millet (Han et al. 2016), and *Pseudomonas fluorescens* of cucumber (Zhou et al. 2017a). However, in light of foxtail millet downy mildew, little information is known on the role of rhizosphere microbial community in pathogen suppression.

Plant genotypes are closely related to their rhizosphere soil microbial composition. Plants provide nutrients and energy for the growth of soil microorganisms by producing root exudates. It is found that different crop rotational systems can form different rhizosphere microbial communities in the same plot (Frindte et al. 2020). Therefore, the diversity and richness of plant rhizosphere soil microorganisms are closely related to crop genotypes. Gu et al. finds that with the continuous planting of Chinese chives, the abundance of pathogenic fungi *Fusarium* has increased, while the abundance of beneficial bacteria *Pseudomonas* significantly has decreased in the rhizosphere soil (Gu et al. 2020).

Likewise, the abundance of *Fusarium*, *Thelephora*, *Hortaea* and *Penicillium* in the rhizosphere soil of the three-year continuous cropping system of *Casuarina equisetifolia* increased year by year (Zhou et al. 2019). Under the strawberry continuous cropping system, the increase of *Fusarium*, *Humicola* and *Arthrotrix* in the strawberry rhizosphere soil has caused the accumulation of phenolic substances, which in turn inhibit the growth of soil-beneficial bacteria *Bacillus*, *Sphingomonas* and *Sphingopyxis* (Chen et al. 2020a). Other studies have also shown that the rotational cropping system can alleviate continuous cropping obstacles by changing the diversity and richness of microorganism and inhibiting the proliferation of pathogenic bacteria (Chen et al. 2020a). An example is that the tomato-celery-cucumber-Chinese cabbage rotational cropping system increases the *Flavobacterium* and *Ohtaekwangia* in the cucumber rhizosphere soil, which effectively alleviate the *Fusarium* wilt of cucumbers infected by *Fusarium oxysporum* f.sp. *cucumerinum* Owen (Zhou et al. 2017a). Soybean-oilseed rape rotation system increases the abundances of *Sphingomonas*, *Bacillus*, *Streptomyces* and *Trichoderma* in oilseed rape rhizosphere soil, and inhibits the clubroot disease caused by *Plasmodiophora brassicae* (Yang et al. 2020). However, for foxtail millet downy mildew, little information is known for the role of rhizosphere microbial community in pathogen suppression. Research on the composition of

rhizosphere microbial community under the Si–Gm–St rotational cropping system can contribute to the discovery of potential microorganisms related to the downy mildew suppression. Therefore, the relationship between the composition of rhizosphere microbial community and downy mildew suppression under foxtail millet and different crop rotational cropping systems deserves further study.

In addition to microbial communities, soil enzymes also act as pivotal parts in the transformation of nitrogen, phosphorus, potassium and organic matter in soil (Pignataro et al. 2012; Burns et al. 2013). The studies of soil enzyme activity can indirectly characterize the physical and chemical properties of soil. Soil enzyme activities can be used as an important indicator in the evaluation of soil quality and fertility. Activities of polyphenol oxidase and urease are significantly positively correlated with the resistance of eggplant to Verticillium wilt (Zhou et al. 2012). And soil microbes greatly influence soil enzyme activities (Han et al. 2016). Previous reports have shown that the enhancement of bacterial diversity increased the enzyme activity in rhizosphere soil of apple trees (Sun et al. 2014a, b). All these indicate that high soil quality and good pathogen-inhibiting ability are closely related to high enzyme activity (Chen et al. 2021).

There are few reports on rotational cropping systems alleviating the obstacles to foxtail millet continuous cropping system, and even fewer studies have reported on the relationship between the changes of soil microbial community and foxtail millet downy mildew susceptibility. In the present study, we used high throughput Illumina sequencing method to compare the microbial communities of foxtail millet rhizosphere soils under the continuous and rotational cropping systems. The purpose of the study is to reveal the relationship between the rhizosphere microbial community and downy mildew disease incidence, and to explore specific fungi and bacterial taxa which can be used for suppressing this disease in the future.

Methods and materials

Experimental design and sample collection

Research plots were established at the southeast of Changzhi City, Shanxi Province, China (E 113°06' N 36°11'), for the purpose of developing a long-term site for studying the 7-year continuous and rotational cropping systems. Soil type at the site is sandy clay loam. This site was planted with maize prior to establish our study. The basic soil properties were pH 7.24, soil organic matter (SOC) 17.63 g kg⁻¹, available nitrogen (AN), available phosphorus (AP) and available potassium (AK) 26.98, 16.21, and 95.60 mg kg⁻¹, respectively. The varieties of crops in this study were Changnong 35 foxtail millet, Zhonghuang 13 soybean (*Glycine max*

(Linn.) Merr.) and Jinshu 16 potato (*Solanum tuberosum* L.). Two different cropping systems were foxtail millet continuous system and foxtail millet-soybean-potato (Si–Gm–St) rotational cropping system during growing season from 2014 to 2020. The area of the plot is 60 m² (15 m × 4 m). There were three replicates for each experimental treatment.

Different crops were planted according to the following densities: plant spacing and row spacing of foxtail millet, soybean and potato were 10 cm × 20 cm, 20 cm × 40 cm and 25 cm × 65 cm, respectively. In 2020, seeds of foxtail millet were randomly sown in field plots under foxtail millet continuous and Si–Gm–St rotational cropping systems. Twenty healthy plants in each plot were randomly selected and sampled on July 7, 2020. First, loose soil adhered to the foxtail millet roots was gently shaken off. Then, the soil tightly adhered to the roots was brushed down and considered as the rhizosphere soil. The rhizosphere soils collected from twenty foxtail millet plants were evenly mixed as a sample. After passing through a 0.5 mm sieve, the mixed sample was divided into three parts for DNA extraction, enzyme activity determination and chemical property analysis, respectively.

Determination of soil chemical properties

Soil pH was measured by an IQ150/pH meter (Spectrum1 Technologies Inc., Aurora, IL, USA) at soil/water 1:5. 10 g of soil was treated with 2 M potassium chloride solution, and then a continuous flow analyzer (Skalar Sanþþ, Skalar Co., The Netherlands) was used to determine the available nitrogen (AN) (Mulvaney 1996). AP and AK were determined by Olsen's method (Olsen and Dean 1982) and flame atomic absorption spectrophotometry, respectively. The dichromic acid oxidation method of Walkley and Black (1934) was used to measure SOC. Soil microbial biomass carbon (MBC) was extracted by chloroform-K₂SO₄ fumigation with a correction factor of 0.33 (Doan et al. 2013; Lipiec et al. 2015). Soil microbial biomass nitrogen (MBN) was measured using the method of Brookes et al. with a correction factor of 0.54 (Brookes et al. 1985).

Foxtail millet downy mildew incidence survey

The determination of foxtail millet downy mildew incidence was carried out during the ear formation period. The healthy or diseased foxtail millet plants were identified based on their visual symptoms. The diseased foxtail millet showed 2–3 pieces of heart leaf tissue were split into filaments that were curved like hair without heading, or the heading was deformed into a hedgehog shape. A large amount of yellow–brown powder (pathogenic oospores) was scattered in the diseased tissue. The foxtail millet downy mildew incidence in each plot was calculated according to the following

formula: number of infected foxtail millet/ number of total foxtail millet × 100%.

Leaf photosynthesis and antioxidant enzymes activity measurement

During August, the net photosynthetic rate of the foxtail millet flag leaves was measured using the LI-6400 photosynthesis assay system (Li-Cor Inc., Lincoln, NE, USA). Then, the foxtail millet flag leaves were collected for enzyme activity determination. Superoxide dismutase (POD) activity was assayed based on the oxidation of guaiacol using H₂O₂ as described by the method of Hammerschmidt et al. (1982). Polyphenol oxidase (PPO) activity was assayed with the method of Yan et al. (2014).

Soil enzyme activity measurement

The activity of urease (UR) and sucrase (SUC) was measured as described by Yao and Huang (Yao and Huang 2006). Phosphatase (PP) activity was carried out as described by Tabatabai and Bremner (Tabatabai and Bremner 1969). Polyphenol oxidase (PPO) activity was determined using the method of Guan (Guan 2020).

Soil DNA extraction, Illumina sequencing and data processing

Soil microbial genomic DNA was extracted from each sample using an E.Z.N.A.®Soil DNA Kit (Omega, Carlsbad, CA, USA). Soil bacterial and fungal communities were sequenced with Illumina Miseq technology. Primer sets of F338 (5'-ACTCCTACGGGAGGCAGCAG-3')/R806 (5'-GGACTACHVGGGTWTCTAAT-3') and ITSF ((5' GGA AGTAAAAGTCGTAACAAGG-3')/ITSR (5'-GCTGCG TTCTTCATCGATGC-3') were applied to amplify the bacterial 16S rDNA gene (V3–V4) and the fungal ITS gene, respectively. The PCR protocol was 4 min denaturation at 94 °C 30 s, followed by 25 cycles of 94 °C 30 s, 50 °C annealing 45 s, 70 °C elongation 30 s, and an extension at 70 °C for 4 min.

The PCR amplification products were purified by a rapid Gel Extraction Kit (Qiagen, Valencia, CA, USA). Bar-coded V3–V4 of bacteria and ITS of fungi amplicons were sequenced with 7 cycle index reads on the Illumina Miseq platform. Sequence reads including ambiguous bases, or average phred score less than 25, or homopolymer run more than 6, or sequence lengths less than 150 bp, or mismatches in primers were eliminated. Only sequences without any mismatches and overlap more than 10 bp in length were assembled. The effective sequences from each sample were uploaded to QIIME for further analysis at 97% similarity of operational taxonomic units (OTU).

The Ribosomal Database Project (RDP) classifier was used to conduct the taxon-dependent analysis (Lan et al. 2012). USEARCH 6.1 in QIIME was used to identify and remove chimeric sequences (Caporaso et al. 2010). Singleton OTUs was eliminated after quality control filtered reads, resulting in an average of 50,531 quality bacterial sequences and 47,038 quality fungal sequences (Table 2). The average read lengths of bacterial 16S rDNA genes and fungal ITS regions were 438 bp and 252 bp, respectively. The accession numbers for bacterial and fungal sequencing data entered at NCBI are PRJNA825072 SRP368639 and PRJNA825114 SRP368847, respectively.

Quantitative PCR

A StepOnePlus™ real-time PCR system (Thermo) was used to estimate the abundance of total bacteria, fungi, *Pseudomonas* and *nifH* in foxtail millet rhizosphere soil. For total bacteria and fungi, forward primer (5'-GGGTTGCGCTCG TTGC-3')/reverse primer (5'-ATGGYTGTCGTCAGCTCG TG-3') and forward primer (5'-GGGTTGCGCTCGTTGC-3')/reverse primer (5'-ATGGYTGTCGTCAGCTCGTG-3') were used for amplification, respectively. The abundance of *Pseudomonas* spp. and *nifH* were amplified using the primers PsF (5'-ACTTAAAGTTGGGAGGAAGGG-3') / PsR (5'-ACACAGGAAATTCACCACCC-3') and *nifHF* (5'-AAAGGYGGWATCGGYAARTCCACCAC-3')/*nifHR* (5'-TTGTTSGCSGCRATCATSGCCATCAT-3'), respectively. Primer sets of total 16S rDNA, fungal ITS, *Pseudomonas* spp. and *nifH* gene were designed and synthesized by Wc gene Biotechnology Corporation (Shanghai, China). Each assay was repeated three times. Assay results were expressed as log₁₀ values (target copy number g⁻¹ soil) for statistics and analysis.

Taxonomy classification and statistical analysis

Abundance counts at the genus level were log₂ transformed and then normalized (Zhao et al. 2013). The Chao and Shannon diversity indices and rarefaction curves were evaluated according to the method of Yu et al. (2015). OTU comparisons of soil samples were presented using a Venn diagram. Column charts were drawn using Sigmaplot, and * indicates significant difference ($P < 0.5$). SPSS 22.0 software was used for independent sample t-test to analyse the significance of differences between two samples (Chicago, IL, USA). Redundancy analysis (RDA) was performed using Canoco 5.0 and the Monte Carlo permutations test (499 permutations) was used to confirm whether soil microbial community compositions were correlated with the soil enzyme activities and properties.

Results

Soil chemical properties, MBC, MBN, foxtail millet disease incidence and yield

Soil chemical properties and microbial biomass were changed under the two different cropping systems (Table 1). Compared with foxtail millet continuous cropping system, AN, AP, AK, SOC, MBC and MBN were significantly increased under the Si-Gm-St rotational cropping system. There was little difference in pH between the two different cropping systems. Under Si-Gm-St rotational cropping system, the foxtail millet downy mildew incidence was 3.00%, which was significantly lower than that under foxtail millet continuous cropping system (15.33%) (Table 1). Furthermore, foxtail millet yields of the two different cropping systems were determined. Table 1 showed that foxtail millet continuous cropping system greatly decreased foxtail millet yield. The foxtail millet downy mildew disease resistance under Si-Gm-St rotational cropping system was significantly higher than that under foxtail millet continuous cropping system.

Physiological characteristics of the foxtail millet flag leaves

Furthermore, foxtail millet plant responses to different cropping systems were assessed by Pn and activities of POD and PPO. As shown in Fig. 1, Si-Gm-St rotational cropping system significantly increased Pn. The activity of PPO also showed the same trend (Fig. 1). It was worth noting that after

Table 1 Soil chemical properties, soil microbial biomass and millet yield in millet continuous (C) and rotational (R) cropping systems

Soil chemical properties, MBC, MBN millet incidence and yield	C	R
pH	7.73 ± 0.06	7.93 ± 0.03
AN (mg kg ⁻¹)	40.37 ± 2.56	50.47 ± 3.34*
AP (mg kg ⁻¹)	21.35 ± 1.50	28.71 ± 1.37*
AK (mg kg ⁻¹)	105.17 ± 2.80	127.33 ± 1.76*
SOC (g kg ⁻¹)	17.29 ± 0.47	20.165 ± 0.87*
MBC (mg kg ⁻¹)	218.44 ± 1.26	377.25 ± 6.6*
MBN (mg kg ⁻¹)	29.45 ± 2.32	41.50 ± 0.95*
Millet yield (kg ha ⁻¹)	3097.00 ± 141.22	3892.00 ± 125.97*
Millet incidence (%)	15.33 ± 0.05*	3.00 ± 0.08

Each value (± standard error) is the mean of three independent experiments

Values followed by * are significantly different at $P < 0.05$

AN available nitrogen, AP available phosphorus, AK available potassium, SOC soil organic carbon, MBC microbial biomass carbon, MBN microbial biomass nitrogen

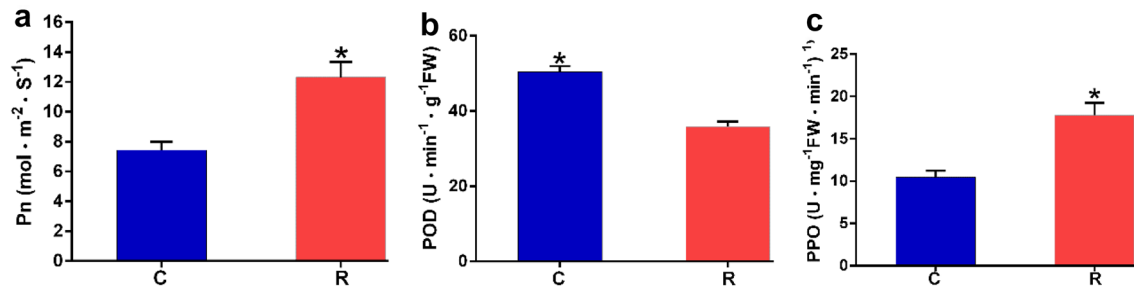


Fig. 1 Net photosynthetic rate (Pn), superoxide dismutase (POD) and polyphenol oxidase (PPO) activities of the millet flag leaves in continuous (C) and rotational (R) cropping systems. Columns labelled with * indicate a significant difference at $P < 0.05$

Si–Gm–St rotational cropping, the PPO activity of foxtail millet increased by 68.80% (Fig. 1). Compared with foxtail millet continuous cropping system, POD activity decreased by 28.92% under Si–Gm–St rotational cropping system.

Soil enzyme activity

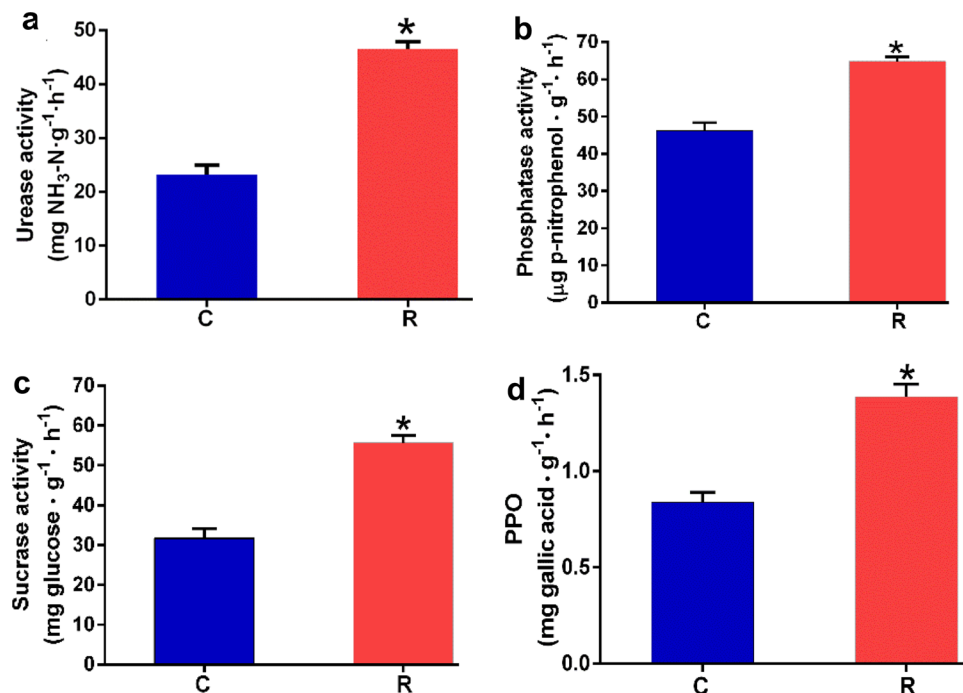
Compared with foxtail millet continuous cropping system, the activities of UR, PP, SUC and PPO were significantly increased by 100.65%, 40.36%, 71.97% and 65.48% under Si–Gm–St rotational cropping system, respectively (Fig. 2).

Soil microbial community richness and diversity

High-throughput Illumina sequencing technology of 16S rDNA/ITS was employed to characterize differences of the bacterial and fungal community compositions in the

foxtail millet rhizosphere soils under two different cropping systems. The rarefaction curve was nearly saturated in all soil samples, implying a reasonable depth and coverage of sequencing (Fig. 3a, b). After quality-filtered and chimera-check, we obtained 48,120 and 52,942 bacteria sequences from foxtail millet rhizosphere soil of foxtail millet continuous and Si–Gm–St rotational cropping system, respectively (Table 2). Fungal sequences were 45,158 and 48,918 under foxtail millet continuous and Si–Gm–St rotational cropping system, respectively (Table 2). Under foxtail millet continuous cropping system and Si–Gm–St rotational cropping systems, the average operation taxonomic unit (OTU) number of bacteria was 4335 and 4450, and the average OTU of fungi was 696 and 733, respectively (Fig. 3c, d). Meanwhile, the diversity indices of bacterial and fungal communities were compared between the two different treatments. As shown in Table 2,

Fig. 2 Activities of urease (UR), phosphatase (PP), sucrase activity (SUC) and polyphenol oxidase (PPO) in the millet rhizosphere soils in continuous (C) and rotational (R) cropping systems. Columns labelled with * indicate a significant difference at $P < 0.05$



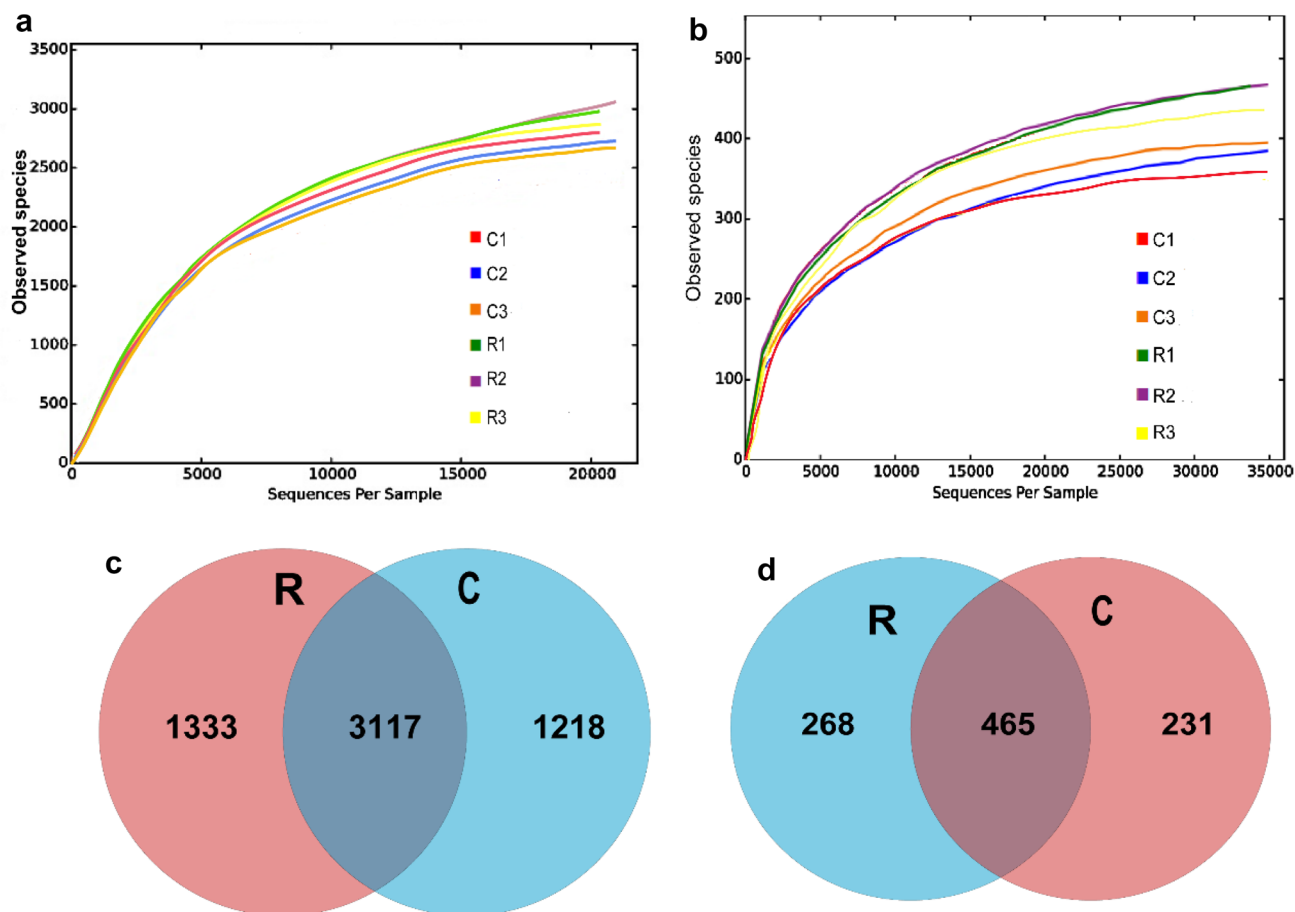


Fig. 3 Rarefaction curves and venn diagram. **a, b** Rarefaction curves for the six soil samples at an OTU threshold of 97% sequence similarity. **c, d** Venn diagram for soil samples collected from millet rhizosphere soils in continuous (C) and rotational (R) cropping systems

Table 2 Number of the sequences and diversity indices based on V3-V4 region of bacterial 16S rDNA genes and fungal ITS region pyrosequencing retrieved from Illumina sequencing in millet continuous (C) and rotational (R) cropping systems

Soil sample	Trimmed sequence	Chao	Shannon
<i>Bacteria</i>			
C	48,120 ± 1566	2438 ± 65	9.71 ± 0.02
R	52,942 ± 822*	3541 ± 129*	9.95 ± 0.7*
<i>Fungi</i>			
C	45,158 ± 8224	427.03 ± 7.73	4.64 ± 0.39
R	48,918 ± 4990*	504.59 ± 14.23*	4.98 ± 0.33*

Each value (± standard error) is the mean of three independent experiments

Values followed by * are significantly different at $P < 0.05$

the alpha-diversity of both bacteria and fungi was higher under Si–Gm–St rotational cropping system than that under foxtail millet continuous cropping system.

Soil bacterial community composition and structure

As shown in Fig. 4a, 17 phyla and subphylum were harboured across all samples with *Alphaproteobacteria*, *Actinobacteria*, *Gemmatimonadetes*, *Chloroflexi*, *Bacteroidetes*, *Acidobacteria*, *Firmicutes*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Betaproteobacteria*, *Nitrospirae*, *Planctomycetes*, *Cyanobacteria*, *Tectomicrobia*, *Verrucomicrobia*, *Saccharibacteria* and *Armatimonadetes* as the major taxa, which occupied more than 99% of the relative abundance of bacteria. However, there were still some variations in relative abundance of each phylum in different samples. Compared with foxtail millet continuous cropping system, the relative abundances of *Chloroflexi*, *Gammaproteobacteria* and *Cyanobacteria* were higher, and the relative abundances of *Bacteroidetes* and *Firmicutes* were lower under Si–Gm–St rotational cropping system ($P < 0.05$) (Fig. 4a). Genera with an average abundance greater than 0.10% in each soil sample were determined as major, and applied in the subsequent analysis. Compared with foxtail millet continuous cropping system, the relative abundances of

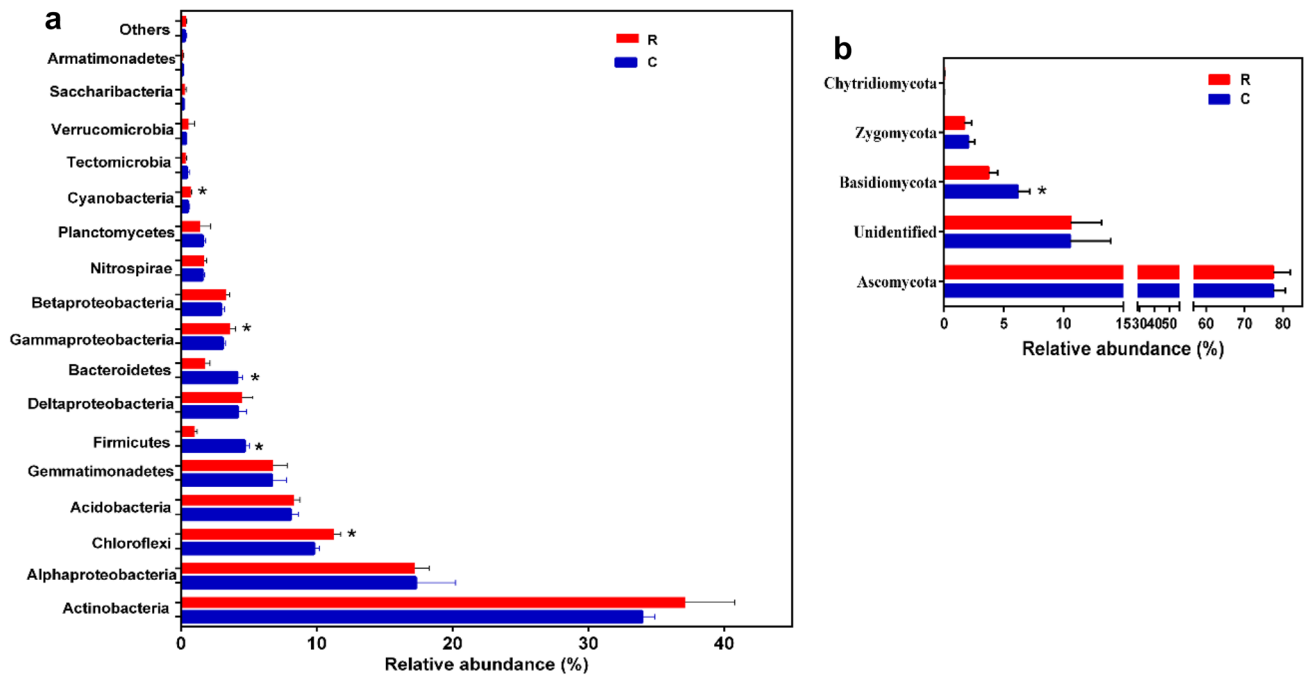


Fig. 4 Relative abundances of the different phyla from the millet rhizosphere soils in continuous (C) and rotational (R) cropping systems. **a** Bacteria. **b** Fungi. Columns labelled with * indicate a significant difference at $P < 0.05$

Nocardioides, *Solirubrobacter*, *Geodermatophilus*, *Devosia*, *Neorhizobium*, *Rubellimicrobium*, *Pseudomonas* and *Ensifer* were higher, while the relative abundances of *Aeromicrobium*, *Rubrobacter*, *Candidatus Entotheonella* and *Achromobacter* were lower under Si–Gm–St rotational cropping system ($P < 0.05$) (Fig. 5a). Pearson’s correlation analysis found that the relative abundances of *Gemmaproteobacteria* and *Chloroflexi* phyla had a significant negative correlation with disease incidence, while an extremely significant positive correlation between *Bacteroidetes* and *Firmicutes* phyla with disease incidence ($P < 0.01$) (Table S1). The relative abundances of *Nocardioides*, *Solirubrobacter*, *Geodermatophilus*, *Devosia*, *Neorhizobium*, *Rubellimicrobium* and *Pseudomonas* had a negative association with disease incidence, while an extremely positive correlation between the relative abundance of *Aeromicrobium*, *Rubrobacter*, *Candidatus Entotheonella* and disease incidence was observed (Table S2).

Soil fungal community composition and structure

Ascomycota and *Basidiomycota* were the dominant phyla in all soil samples, and the relative abundances of these two phyla occupied more than 83% of the fungal sequences. Moreover, the relative abundances of *Zygomycota* and *Chytridiomycota* were more than 1% in at least one sample (Fig. 4b). The foxtail millet continuous cropping system has more relative abundance of *Basidiomycota* than the

Si–Gm–St rotational cropping system ($P < 0.05$). Compared with foxtail millet continuous cropping system, the relative abundances of *Mortierella*, *Ascobolus*, *Conocybe*, *Cyathus*, *Rhizophlyctis*, *Podospora* and *Acremonium* were higher and the relative abundances of *Myrothecium*, *Corynespora*, *Thermoascus*, *Pseudogymnoascus*, *Gymnoascus*, *Thermomyces*, *Schizothecium*, *Ilyonectria*, *Cylindrocarpon*, *Fusarium* were lower under Si–Gm–St rotational cropping system (Fig. 5b). A significant positive correlation between the relative abundance of *Basidiomycota* and disease incidence was shown in a Pearson’s correlation analysis ($P < 0.01$) (Table S1). The relative abundances of *Mortierella*, *Acremonium*, *Podospora*, *Conocybe*, *Cyathus* and *Ascobolus* genera had negative association with disease incidence, while an extremely positive correlation between disease incidence and *Fusarium*, *Cylindrocarpon*, *Ilyonectria*, *Pseudogymnoascus*, *Thermoascus* and *Myrothecium* was observed ($P < 0.01$) (Table S2).

Correlation analysis

The effects of soil chemical properties on microbial community composition were investigated using RDA analysis (Fig. 6). Figure 6a showed that the axe1 and axe2 accounted for 55.57% and 24.42% of the distribution of soil bacterial community structure at the phylum level. SOC, AN, AP and AK had the highest correlation with *Betaproteobacteria*, *Gammaproteobacteria*, *Cyanobacteria* and *Chloroflexi*.

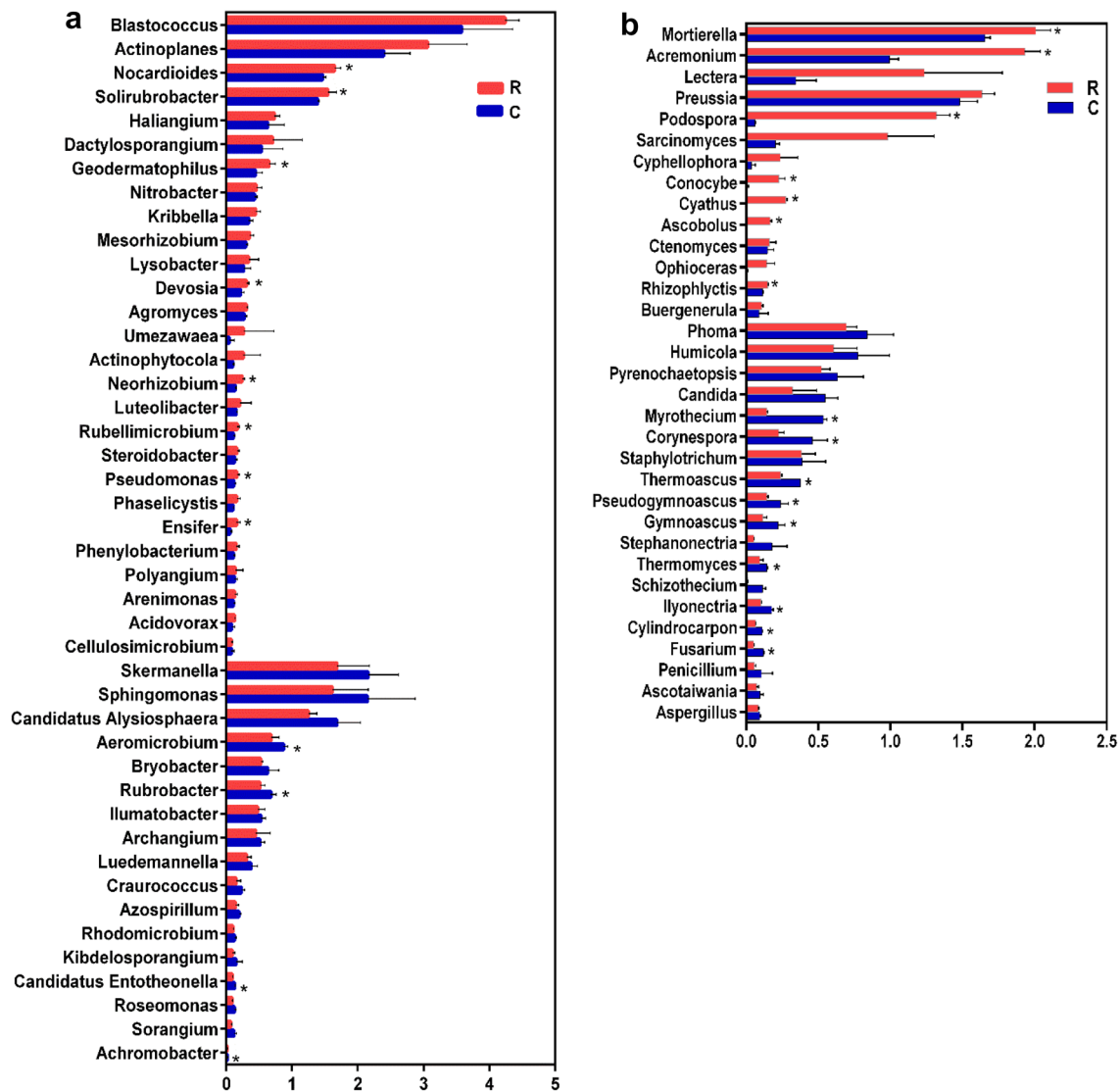


Fig. 5 Relative abundances of the different bacterial and fungal genera from the millet rhizosphere soils in continuous (C) and rotational (R) cropping systems. **a** Bacteria. **b** Fungi. Columns labelled with* indicate a significant difference at $P < 0.05$

Figure 6c showed that the *axe1* and *axe2* accounted for 70.26% and 12.57% of the distribution of soil bacterial community structure at the genus level. The *Nocardioides*, *Solirubrobacter*, *Geodermatophilus*, *Devosia*, *Neorhizobium*, *Rubellimicrobium*, *Pseudomonas* and *Ensifer* genera had a positive correlation with SOC, AN, AP and AK. Figure 6b showed that the *axe1* and *axe2* accounted for 80.72% and 3.85% of the distribution of fungal community structure at the phylum level. SOC, AN, AP and AK were positively correlated with *Cercozoa* and *Chytridiomycota*. Figure 6d showed that the *axe1* and *axe2* explained 75.59% and 16.75% of the distribution of fungal community structure at the genus level. The *Fusarium*, *Acremonium*, *Cyphellophora*, *Ophioceras* and *Preussia* genera had a positive correlation

with SOC, AN, AP and AK (Fig. 6d). AN, AP, AK and SOC significantly affected the distribution of fungal communities (Fig. 6b, d). At the genus level, soil chemical properties significantly affected the distribution of bacterial communities (Fig. 6c). The effect of soil enzyme activities on soil chemical properties was shown in Fig. 6e. The *axe1* and *axe2* accounted for 96.30% and 0.55% of the changes of chemical properties (Fig. 6e). UR, PP, SUC and PPO were significantly correlated with SOC, AN, AP and AK.

Soil microbial abundances

Compared with foxtail millet continuous cropping system, the abundances of soil bacteria, *Pseudomonas* spp.

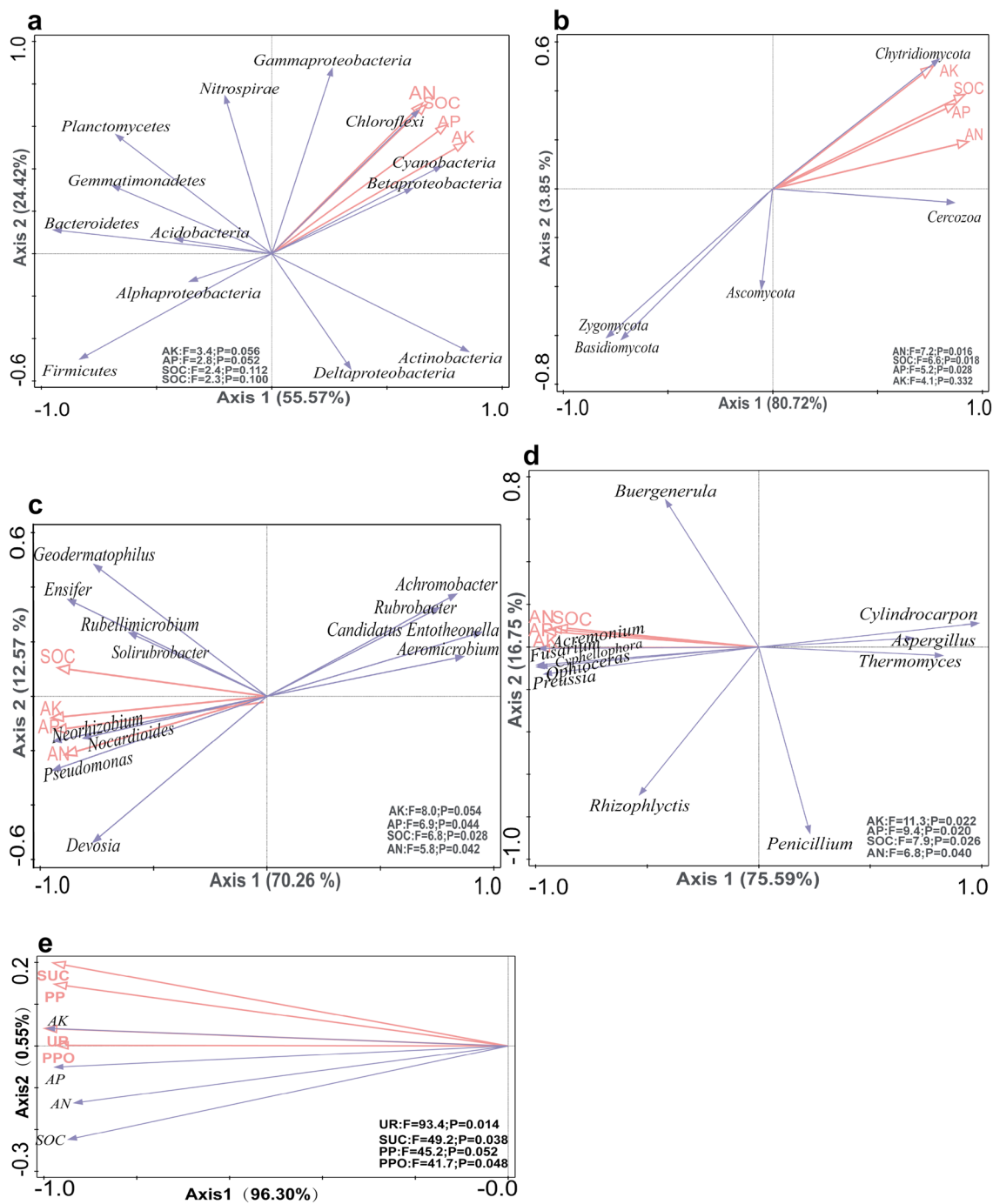


Fig. 6 Redundancy analysis depicting the relationship between the soil properties, soil enzyme activities and the abundance of bacterial and fungal taxa. The genera with more than 0.5% average abundance in at least one sample were used

and *nifH* were 2.71, 2.40 and 1.82 times than that under Si–Gm–St rotational cropping system, respectively (Table. 3). Bacteria/Fungi value was significantly higher

under foxtail millet continuous cropping system than that under Si–Gm–St rotational cropping system ($P < 0.05$) (Table 3).

Table 3 Soil microbial abundances in millet continuous (C) and rotational (R) cropping systems

Soil sample	Bacteria 10 ⁹ copies g ⁻¹ soil	Fungi 10 ⁸ copies g ⁻¹ soil	<i>Pseudomonas</i> spp. 10 ⁷ copies g ⁻¹ soil	<i>nifH</i> 10 ⁵ copies g ⁻¹ soil	Bacteria/Fungi
C	1.54 ± 0.10	1.0 ± 0.02*	1.26 ± 0.07	4.13 ± 0.07	2.40 ± 0.02
R	4.17 ± 0.03*	0.64 ± 0.07	3.03 ± 0.28*	7.51 ± 0.21*	4.17 ± 0.03*

Each value (± standard error) is the mean of three independent experiments

Values followed by * are significantly different at $P < 0.05$

Discussion

Si–Gm–St rotational cropping system improves foxtail millet physiological indicators and rhizosphere microbial richness and diversity

Rhizosphere microbial community composition and structure of healthy plant can increase plant disease resistance and maintain plant health. Studying plant microbial community structure of rhizosphere soil can provide new ideas for exploring functional microorganisms that might inhibit soil-borne plant pathogens (Schweitzer et al. 2008). In agricultural production, different crop rotational systems can alleviate the spread of soil-borne diseases caused by crop continuous cropping systems. Qin et al. found that after potato rotation with oat or faba bean, the incidence of tuber black scurf decreased the abundances of beneficial microorganisms in potato rhizosphere soil and potato yield (Qin et al. 2022). Xu et al. showed that compared with foxtail millet-maize, foxtail millet-potato-maize and foxtail millet-maize-soybean rotational systems, the Si–Gm–St rotational system harbored the highest foxtail millet yield and lowest downy mildew incidence (Xu et al. 2022). Thus, we investigated the microbial community composition of foxtail millet rhizosphere soils under foxtail millet continuous and Si–Gm–St rotational cropping systems. Compared with foxtail millet continuous cropping system, Pn and antioxidant enzyme activities of foxtail millet flag leaves were changed under Si–Gm–St rotational cropping system (Fig. 1). The composition of nutrients and secondary metabolites excreted by plant roots may be affected by these physiological changes, which in turn alter plant rhizosphere microbial community composition and structure (Han et al., 2016). A previous report showed that the bacterial community composition in the rice rhizosphere soil changed after rice-maize rotation (Maarastawi et al. 2018), which is consistent with our findings under Si–Gm–St rotational cropping system (Table 3). Similarly, the richness and diversity of beneficial bacteria significantly increased in the tomato rhizosphere soil under tomato-celery rotational cropping system (Jin et al. 2021). Plant genotype has an important effect on the microbial community compositions of rhizosphere soil. Plants can produce root exudates and exfoliants,

which provide nutrients and energy for the growth of soil microorganisms. Different plant genotypes can stimulate or inhibit the growth of specific microorganisms in their rhizosphere soils by secreting different compounds (Han et al. 2016). At the same time, rhizosphere soil microbes can also affect plant growth by changing the soil micro-ecological environment. Our result showed that the composition of rhizosphere bacterial and fungal communities varied greatly under different cropping systems (Figs. 4, 5). These differences may be caused by the rotation of soybean and potato with foxtail millet. Previous research found that the bacterial OUT of cucumber rhizosphere soil increased under tomato-celery-cucumber-Chinese cabbage rotational cropping system, which also proved our findings (Zhou et al. 2017a). Many studies have shown that the distribution of high abundance and diversity of functional bacteria is closely related to disease suppression (Rosenzweig et al. 2012; Wu et al. 2015). The results of high-throughput sequencing showed that the Chao and Shannon values of bacteria in foxtail millet rhizosphere soil were significantly increased under Si–Gm–St rotational cropping system, and the number of effective sequences also significantly increased (Table 2). The higher diversity of bacterial community composition, the more stable rhizosphere ecosystem can be provided for plants, which will be beneficial to promote crop growth and resist the invasion of plant soil-borne pathogens (Chen et al. 2020b). Moreover, the enrichment of antagonistic microorganisms in plant rhizosphere soil usually helps to improve plant disease resistance. Therefore, we conclude that higher rhizosphere antagonistic bacterial diversity in our study might be beneficial for suppressing foxtail millet downy mildew suppression.

Si–Gm–St rotational cropping system improves diversity of functional bacteria in foxtail millet rhizosphere soil

We also conducted an in-depth study on the species of bacterial community composition of foxtail millet rhizosphere soil under foxtail millet continuous and Si–Gm–St rotational cropping systems. Compared with foxtail millet continuous cropping system, large amounts of *Cyanobacteria* and *Gemmaproteobacteria* were observed under Si-Gm-St rotational

cropping system (Fig. 3). Species of *Cyanobacteria* phylum were mainly involved in the fixation of free nitrogen in agricultural soils (Zhou et al. 2017b). The relative abundance of *Gammaproteobacteria* in foxtail millet rhizosphere soil increased under Si–Gm–St rotational cropping system (Fig. 4). Other results showed that *Gammaproteobacteria* was copiotrophic bacteria and suitable for proliferation in soil with high content of SOC and AN (Fierer et al. 2007; Fierer et al. 2012). In our results, there were higher levels of AN, *nifH* and SOC under Si–Gm–St rotational cropping system (Tables 1, 3), which supported this conclusion. Likewise, *Gammaproteobacteria* was enriched in corn-rice rotational cropping soil than in corn or rice continuous cropping soil in the Philippine (Maarastawi et al. 2018). Moreover, the relative abundances of *Firmicutes* and *Bacteroidetes* were increased in foxtail millet rhizosphere soil under foxtail millet continuous cropping system compared with Si–Gm–St rotational cropping system. The difference in relative abundances of *Cyanobacteria*, *Gammaproteobacteria*, *Bacteroidetes* and *Firmicutes* indicated that these four phyla were closely related to the occurrence of foxtail millet downy mildew incidence (Table S1). To further study on the relationship between bacterial taxa and disease occurrence, we performed a genus level analysis. We speculate that the increase of bacterial diversity under Si–Gm–St rotational cropping system might be related to the higher nutrient level in soil and foxtail millet downy mildew suppression. Xu et al. found that higher AN and SOC were conducive to the distribution of bacterial diversity in the soil (Xu et al. 2018). *Neorhizobium* is a non-symbiotic neorhizobia isolated from dryland soils (Soenens et al. 2018). These non-nodular endophytes colonize root nodules can maintain a good micro-ecological environment between plants and soil microorganisms. It has been reported that *Devosia netuniae* contains the *nifH* gene and can form a unique nitrogen-fixing foot nodule symbionts with the aquatic legume *Neptunia natans* (L.f.) Druce. (Rivas et al. 2002). Chen et al. found that intercropping of peanut and maize increased the abundance of *nifH* compared with monoculture maize (Chen et al. 2017). Combining these reports with our results, we conclude that Si–Gm–St rotational cropping system may be more suitable for the proliferation of nitrogen-fixing bacteria. Other studies have shown that some species of the genera *Neorhizobium* and *Devosia* may participate in the nitrogen cycle in the soil, increasing the level of AN. However, in order to better demonstrate the nitrogen fixation mechanism of *Neorhizobium* and *Devosia*, related in-vitro experiments are still needed. *Nocardioide*s degraded carbendazim and other organic compounds containing benzene ring (Busato et al. 2016). Our study found an enrichment of *Pseudomonas* spp., members of which are known to be endophytic bacteria that promote plant growth. For example, *Pseudomonas fluorescens* has been used to promote the plant growth by

producing 2, 4-diacetylphloroglucinol (Landa et al. 2003). *Pseudomonas koreensis* group strains were dominated in the cocoyam rhizosphere and can suppress *Pythium* root rot by producing cyclic lipopeptide (Qin et al. 2022). Other results showed that *Pseudomonas* spp. was involved in the decomposition of alcohols, ethers and phorate in soil, and could antagonize soil pathogens such as *Rhizoctonia solani* Ag3 (Garbeva et al. 2004). In addition, q-PCR results found that the abundance of *Pseudomonas* spp. was increased under Si–Gm–St rotational cropping system, which further verified the conclusion of soil microbial diversity analysis (Table 3). Meanwhile, the correlation analysis showed that *Pseudomonas* spp. had a negative correlation with the disease incidence (Table S1). *Aeromicrobium* isolated from ginseng rhizosphere soil can produce erythromycin, a macrolide antibiotic with antibacterial function. Therefore, our results combined with earlier studies suggested that *Pseudomonas* spp. and *Aeromicrobium* might play important roles in the suppression of downy mildew pathogen. However, future studies such as in vitro antifungal tests using isolated *Pseudomonas* spp. and *Aeromicrobium* bacteria, are still needed to identify their antagonistic activities against smut disease pathogen and better understand the relative mechanisms.

Si–Gm–St rotational cropping system improves diversity of functional fungi in foxtail millet rhizosphere soil

In addition, studies on the fungal community composition showed that *Basidiomycota* was increased in foxtail millet continuous cropping system. This is consistent with Wu's research results in the rhizosphere soil of ginseng continuous cropping system (Wu et al. 2019). Leite et al. found that *Basidiomycota* has the ability to degrade cellulose (Leite et al. 2017). Some species of *Basidiomycota* phylum cultures secrete exopolysaccharides with strong antioxidant properties and immunity, good antibacterial properties and antitumor activity (Osińska-Jaroszuk et al. 2015). RDA analysis showed that SOC, AN and AP were significantly positively correlated with the distribution of soil fungal diversity at the genus level (Fig. 6d). Some *Mortierella* spp. could promote plant growth by producing antibiotics against various plant pathogens (Xiong et al. 2017; Liu et al. 2020a). *Fusarium graminearum* could infect the roots of maize seedlings (Zhao et al. 2013). *Fusarium oxysporum* f.sp. *niveum*. was the pathogen of watermelon rhizosphere *Fusarium* wilt. Further studies showed that *Fusarium* spp. could colonize asparagus rhizosphere and increase the disease incidence (Gilardi et al. 2013). In this study, the increased abundance of *Fusarium* may be related to the enhancement of soil borne diseases in the rhizosphere of *Rehmannia glutinosa*. *Ophioceras* was suitable for living in soils with high nitrogen levels and had the function of breaking down carbohydrates (Garrett

et al. 2010). *Cyathus striatus* could produce highly active extracellular enzymes that promoted the cycling of carbon, nitrogen and phosphorus in soil (Wang et al. 2014). *Acremonium coenophialum* could increase the productivity and stress tolerance of tall fescue (Belesky and Fedders 1995; Martínez-culebras et al. 2004). Thermostable hemicellulase produced by *Thermomyces lanuginosus* may contribute to hemicellulose degradation during composting (Zhang et al. 2015). The main functions of the above microbial groups were concentrated in the decomposition and denitrification of cellulose, thereby promoting the cycle of carbon and nitrogen. It was also the reason for the decreasing of AN and SOC in foxtail millet continuous cropping system. It was found that the number of fungi could participate in soil nutrient cycling increased, while some pathogenic fungi decreased under Si–Gm–St rotational cropping system. This indicated that Si–Gm–St rotational cropping system could reduce the spread of soil borne diseases.

Si–Gm–St rotational cropping system increases the activity of nutrient cycling enzymes in foxtail millet rhizosphere soil

The Si–Gm–St rotational cropping system increased above-ground plant diversity, and the residues and root exudates of different plant genotypes enhanced soil nutrients and microbial diversities (Liu et al. 2020b). The abundant soil microbes can increase the enzyme activities of rhizosphere soil. Otherwise, the increased enzyme activities promoted the decomposition of nutrients in the soil, thereby increasing the microbial diversities in the soil (Ndabankulu et al. 2022). For example, soil alkaline phosphatase plays an important role in the mineralization of organic matter, which can convert phosphorus components in soil into available inorganic phosphorus, thereby increasing the diversity of soil microorganisms (Ullah et al. 2019). In addition, the other studies also show that PP activity in the rhizosphere soil of resistant banana varieties is higher than that of susceptible varieties (Sun et al. 2013). Compared with foxtail millet continuous cropping, higher nitrogen levels increased soil microbial diversity under crop rotation treatments, which in turn promotes the activities phosphatase and urease (Treseder and Vitousek 2001). The hydrolysis of sucrose by sucrase can reflect the conversion ability of SOC, and the catabolism of sucrose can provide a carbon source for the growth and reproduction of microorganisms (Cantarella et al. 2018). PPO activity is positively correlated with plant disease resistance. PPO is an important oxidoreductase, which plays an important role in soil antibacterial defense system (Zhou 2012). Furthermore, millet rhizosphere soil of the Si–Gm–St rotational system harbored higher enzyme activities as compared to the millet continuous cropping system (Fig. 2). The reason may be that the increase of rhizosphere microbial

community diversity is closely related to higher soil enzyme activity (Sun et al. 2014a, b). Some specific bacterial and fungal taxa may support effective nutrient cycling, and can resist soil diseases to a certain extent, and thus to provide better nutritional conditions for foxtail millet growth under Si–Gm–St rotational cropping system.

Conclusions

Compared with foxtail millet continuous cropping system, the diversities and abundances of bacteria and fungi were increased, the foxtail millet downy mildew incidence was reduced, and the yield was increased under Si–Gm–St rotational cropping system. Downy mildew suppression may be associated with high bacterial diversity and enzyme activity in the rhizosphere soil. Furthermore, the specific rhizosphere microbial community was composed, especially with the high abundance of beneficial bacteria *Pseudomonas* and *Aeromicrobium* and low abundance of pathogenic fungi *Fusarium*. The two genera *Pseudomonas* and *Aeromicrobium* could be used as the potential bacteria to suppress downy mildew in the future, although their antagonistic activities against the obligate parasitic oomycetes pathogen still need to be further identified. In conclusion, the changes of soil microbial community structure and function are jointly restricted and influenced by Si–Gm–St rotational cropping and soil environmental factors. Our findings provide a basis for further development of sustainable and environmentally compatible agricultural systems under Si–Gm–St rotational cropping system.

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Declarations

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