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REGULAR ARTICLE

# Changes in the soil microbial community are associated with the occurrence of *Panax quinquefolius* L. root rot diseases

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**Abstract**

*Background and aims* Root-rot disease, a catastrophic disease of *Panax quinquefolium* L. causes yield reduction and serious economic losses. However, knowledge of the relationship between rhizosphere microbial community and root-rot disease is limited. This study is aim to test whether the bacteria and fungi community differed between the soil attached to healthy and rotten roots of American ginseng. Moreover, the effects of American ginseng cultivation for 4 years on changes of soil physiochemical properties and microbial community were also investigated.

*Methods* High-throughput sequencing (Illumina MiSeq) was used to investigate the difference of microbial communities in the soils of new farmland (C) and the rhizosphere soils around healthy (H) and root rot diseased ginseng (R).

*Results* Cultivation of American ginseng for 4 years not only changed the soil physicochemical properties, but also significantly increased the richness of the soil bacteria and decreased the fungal richness and diversity. Compared with other genera, the bacterial genera *Nitrospira* and the fungal genera *Gibberella* and *Podospora* were strongly enriched in the soil of new farmland. However, the relative abundance of *Janthinobacterium, Nitrospira* and *Pedomicrobium* in bacterial community, and *Mrakia, Paradendryphiella, Sporopachydermia, Myrothecium* and *Racocetra* in fungal community were significantly decreased after culture of American ginseng. The results also showed that the bacteria and fungi community differs between the soil attached to healthy and rotten roots of American ginseng. The richness indices of fungal community showed a significant decrease in rhizosphere soils of R comparing with H. The bacteria *Rhodoplanes* and *Kaistobacter* were the dominant genera in the H sample, whereas *Sphingobium* was dominant in the R sample. Notably, *Monographella* was significantly higher in the R sample (23.13%) than that of H sample (2.90%). In addition, the fungi *Melanophyllum* and *Staphylotrichum* were the most differently abundant in the H sample, whereas *Mortierella* and *Cistella* were the differently abundant genera in the R sample.

*Conclusions* Our results indicate that cultivation of American ginseng changed the edaphic factors and the soil microbial community, and there are significant differences in the microbial community between the soil attached to healthy and rotten roots of American ginseng.

Jinglong Jiang and Miao Yu contributed equally to this work.

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**Keywords** American ginseng · Root rot diseases · Community diversity · Illumina MiSeq sequencing

**Abbreviations**

| | |
|---|---|
| *ACE* | Abundance based coverage estimator |
| ANOSIM | Analysis of similarities |
| *ANOVA* | Analysis of variance |
| *CAG* | Cultivate American ginseng |
| *EC* | Electrical conductivity |
| HCN | Hydrogen cyanide |
| *ITS* | Internal transcribed spacer |
| *LDA* | Linear discriminant analysis |
| *LEfSe* | Linear discriminant analysis effect size |
| *OM* | Organic material |
| *OTUs* | Operational taxonomic units |
| *PCoA* | Principal co-ordinates analysis |
| *PCR* | Polymerase chain reaction |
| *Phl* | 2,4-diacetylphloroglucinol |
| *QIIME* | Quantitative insights into microbial ecology |
| *SRA* | Sequence Read Archive |
| *SPSS* | Statistical product and service solutions |
| *UNITE* | unite.ut.ee |
| *WPGMA* | Weighted pair group method with arithmetic averages |

## Introduction

American ginseng, or xiyangshen (*Panax quinquefolius* L.), is a highly valuable herb in the Araliaceae family, as its dry root and root extracts have been popular health supplements in Asia for hundreds of years. Undoubtedly the biggest challenge to American ginseng production is the management of diseases which render the ginseng root unmarketable due to infection primarily by fungal pathogens (Punja 2011). High planting density, over fertilization, and growth condition that are damp and warm with reduced sunlight and air flow all provide a favorable environment for plant pathogens, especially root-rot disease, which would consequently lead to crop yield and quality reduction (Wu et al. 2016). These diseases are primarily caused by fungal pathogens, including several species of *Fusarium* (causing root rot or rusty root) (Bi et al. 2011; Punja et al. 2007; Rahman and Punja 2005), *Rhexocercosporidium panacis* (causing rusty root) (Reeleder 2007), *Cylindrocarpon destructans* (causing root rot) (Punja 1997; Reeleder and Brammall 1994), and *Phytophthora cactorum* (causing Phytophthora root rot) (Punja 1997; Darmono et al. 1991). Among these pathogens, *Fusarium solani* and *F. oxysporum* are highly aggressive fungi causing American ginseng root rot in the Beijing ginseng producing region of China (Bi et al. 2011). Our survey found that root rot of American ginseng has become an important factor limiting the production of American ginseng in the Liuba area, Hanzhong, a major producing area in the Northwest of China. Typical early symptoms of root rot diseases in American ginseng are reddish-brown to orange-brown discolored areas on the root surface (Jiao et al. 2015). Rotten symptoms include dry rot in both exterior and interior root tissues and loss of fibrous roots with the development of disease (Jiao et al. 2015).

Although these pathogens are frequently isolated from the roots of decaying American ginseng, whether these pathogens are dominant microbial communities in the rhizosphere soil has rarely been reported. In addition, the bacterial community in the rhizosphere soil of root rot is often overlooked due to the isolation of fungi from American ginseng. The initiation of root rot has been associated with deterioration of the physicochemical properties of the soil especially in a soil with long-term inorganic fertilization and continuous cropping, autotoxicity, and changes in the soil microbial community (Ogweno and Yu 2006; Huang et al. 2013). Conventional farming practices with time have led to decline in soil structure, fertility and microbial diversity and simultaneously given rise to many soil and root borne diseases (Ahanger et al. 2014). Root diseases are more damaging when soil conditions are poor as a result of inadequate drainage, poor soil structure, low organic matter and low soil fertility (Ahanger et al. 2014). Autotoxicity is a type of intraspecific allelopathy where a plant species inhibits the growth of its own or relatives through the release of toxic chemicals into the environment (Singh et al. 1999; Yu et al. 2000). Some reports illuminated that autotoxins in root tissue of asparagus show synergism with *Fusarium oxysporum* spp. asparagi causing root rot and increasing the incidence of the disease (Hartung and Stephens 1983; Peirce and Colby 1987; Nigh 1990). Soil microorganisms have profound effects on the growth, nutrition and health of plants in natural and agricultural ecosystems (Garbeva et al. 2004; Almario et al. 2013). Among these, imbalances (the increase of potential pathogens and the decrease of beneficial soil organisms) in soil microbial

communities were shown to be the pivotal event for infection of peanuts by root rot-causing microbes (Chen et al. 2012). Tan et al. (2017) investigated the rhizospheric and root endophytic fungi during continuous cropping of sanchi (*Panax notoginseng*), and the result demonstrated that root-rot disease affected the community structure and diversity of rhizospheric and root endophytic fungi. Xiong et al. (2017) researched synthetically the soil microbial communities and analyzed the relative importance of bacterial and fungal communities for the suppression of *Fusarium* wilt through Illumina MiSeq sequencing, and indicated that fungal communities may be important in the development of soil suppressiveness against vanilla *Fusarium* wilt disease. Dong et al. (2017) revealed that the diversity and composition of the soil bacterial and fungal communities changed during the continuous cropping of American ginseng compared to the communities present during cultivation of traditional crops. However, these reports mainly focused on the microbial community of rhizospheric soil during repeated cropping or crop rotation. At this time, it is unknown if there are any differences in the microbial communities in soils that have been planted with American ginseng for 4 years and in soils that have never been used to cultivate American ginseng. Since we also observed that healthy and rotted American ginseng roots are both present in the same land, we speculated that there may be differences in the microbial community of the rhizospheric soil of healthy and diseased ginseng from the same plot.

In this study, we compared the soil physicochemical propertiessoil and microbial communities of barren and cultivated fields. In addition, we also tested whether the rhizospheric soil of healthy and rotten roots differed in bacterial and fungal abundance, diversity and taxonomic composition.

## Materials and Methods

### Soil collection

The sampling site located in Liuba County (a American ginseng research farm) (33° 38' N, 106° 43' E), Hanzhong, Shaanxi province, which is one of the main areas of American ginseng production in China. The mean annual temperature and altitude in this area are 22.1°C and 1, 722 m. In order to investagate the effects of American ginseng cultivation for 4 years on changes of soil physiochemical properties and microbial community, a plot of land that has never been planted in any crop was chosen as the blank control plot (C). This barren land had been treated to prepare for a ginseng crop in accordance with the similar cultivation methods including edaphic treatment, agronomic management and fertilization regimes. Farmland that has been planted in American ginseng (CAG) for 4 years and is 50 m from the control soil was selected for the experimental samples (Fig. 1a). In this farmland, it was found that there were some sporadic lands (about 15%) in which American ginseng did not grow new seedlings on April 30, 2017, and the roots of these places dug out were found to root rot. Soil samples were collected from the four corners and the diagonal center of each plot (five-point sampling method) (C and CAG) (Fig. 1a) to analyze the physicochemical properties. Control soil samples (C) were collected by digging 10-20 cm into the fallow plot for a total of 8-10 g of soil, and soil from the 5 sampling points were random chosen to 3 samples (C1, C2, C3). Rhizosphere soil samples around 5 healthy ginseng (H) and 5 root rot-diseased ginseng (R) were collected by digging them up (Fig. 1c, d) and shaking off the soil into a plastic bag accordling to five-point sampling method. After identification, rhizosphere soil samples of 3 healthy (H1, H2, H3) and 3 diseased ginsengs (R1, R2, R3) were collected for experimental analysis. After excavating the healthy or diseased American ginseng roots, 8 to 10 g of soil were taken from where the ginseng had been growing for determination of the physicochemical properties of the soils in the CAG fields. Samples for microbial testing were placed on ice before transport to the laboratory of Plant Biotechnology Research Center of Shaanxi University of Technology, Shaanxi, China. One portion of each sample was air-dried and refrigerated before chemical and soil property analysis. The other portions were kept in aseptic valve bags, transported in an ice box, and immediately processed upon return to the laboratory.

### Soil physicochemical properties

Three soil parallel samples were collected from the C plot and the CAG plot to measure the physicochemical properties. The samples were homogenized by being passed through a 2-mm sieve. The soil pH was determined using a glass electrode pH meter (Mettler-Toledo FE20-Five Easy PlusTM, Schwerzenbach, Switzerland)

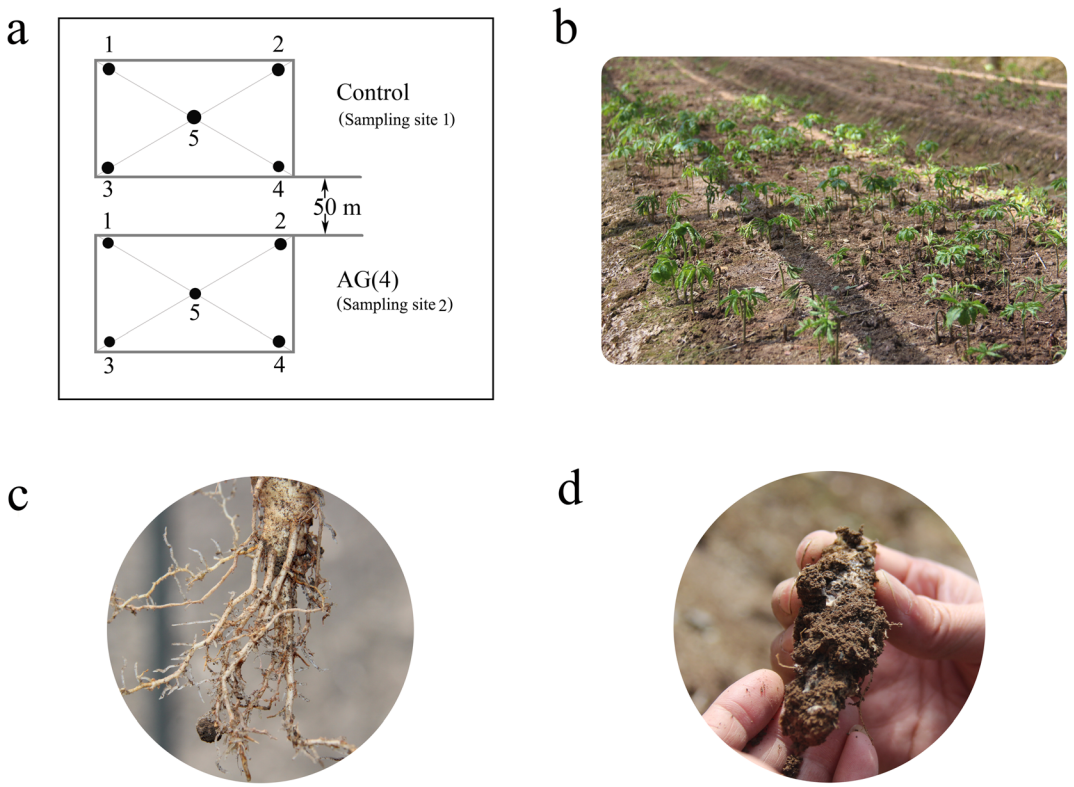


**Fig. 1** Collection of the rhizosphere soil of American ginseng from two sampling sites. Five samples were collected according to the diagonal method (**a**) from a fallow field and a field growing American Ginseng for 4 years (**b**). Rhizosphere soil samples from healthy ginseng (H) and root-rot ginseng (R) were collected by digging up ginseng roots, identifying them as healthy (**c**) or root-rot diseased (**d**), and shaking the soil from the root

in a 1: 2.5 soil:water (w/v) suspension (Qiu et al. 2012). The amount of organic matter was determined by quantifying the amount of oxidized soil carbon based on its reaction to acidic dichromate ($Cr_2O_7^{2-}$). Total nitrogen was measured based on direct combustion using an elemental analyzer (Vario EL III, Germany). The hydrolytic nitrogen in the soil was determined based on the transformation of hydrolyzed nitrogen into ammonia nitrogen through sodium hydroxide (Tan et al. 2017). Total phosphorus was measured by acid digestion (Grimshaw 1987). Total potassium was measured after digesting the soils in a mixture of concentrated nitric acid ($HNO_3$) and perchloric acid ($HClO_4$) using a soil-acid ratio of 1:10 (Tan et al. 2017). Available phosphorus (P) was determined using the sodium hydrogen carbonate solution-Mo-Sb anti-spectrophotometric method (Shen et al. 2011). Soil available potassium (K) was measured using a standard protocol according to McLean and Watson (1985).

## Genomic DNA extraction, PCR amplification and High throughput amplicon sequencing

About 0.25 g of the chilled soil samples were used to extract total genomic DNA using PowerSoil™ DNA Isolation Kits (MoBio Laboratories, Solana Beach, CA, USA) according to the manufacturer's protocol. Total DNA was used to analyze the changes in the microbial communities among the control soil (C), healthy ginseng rhizosphere soil (H), and root rot ginseng rhizosphere soil (R) samples. Genomic DNA concentration and purity were measured using a NanoDrop ND-2000 (NanoDrop Technologies, Wilmington, DE) spectrophotometer. The V3-V4 region of the 16S rRNA gene was amplified using the primer set 338F (5′- ACTCCTACGGGAGGCAGCA -3′) and 806R (5′- GGACTACHVGGGTWTCTAAT-3′) for bacterial community analysis. For fungal community analysis, the Internal Transcribed Spacer (ITS1) sequence, including the partial 18S rRNA gene,

was amplified with primer set ITS 5F (5′-GGAA GTAAAAGTCGTAACAAGG-3′) and ITS 1R (5′-GCTGCGTTCTTCATCGATGC-3′). Sample-specific 7-bp barcodes were incorporated into the primers for multiplex sequencing. The PCR amplification was carried out in a total volume of 25 μL containing 5 μL 5 × reaction buffer, 5 μL 5 × GC buffer, 2 μL dNTPs (2.5 mM), 1 μL forward primer (10 μM), 1 μL reverse primer (10 μM), 2 μL DNA template, 8.75 μL dd$H_2O$ and 0.25 μL Q5 High-Fidelity DNA Polymerase (NEB, USA). The PCR protocol consisted of an initial denaturation step at 98 °C for 2 min, 25 30 cycles of denaturation at 98 °C for 15 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, a final extension at 72 °C for 5 min, and a hold at 10 °C. Three replicates of the amplifications were pooled together to minimize the PCR bias. PCR products were detected by electrophoresis through 2.0% agarose gels and appropriately sized fragments (about 450 bp for bacteria and 350 bp for fungi) were purified with DNA Gel Extraction Kit (Axygen, Union City, CA, USA). PCR amplicons were quantified using the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). Finally, paired-end 2×300 bp sequencing of fungal and bacterial amplicons were carried out on the Illumina MiSeq sequencer at Personal Biotechnology Co., Ltd (Shanghai, China). The detailed experimental procedure is shown in Fig. S1.

## Sequence analysis

After removing the adaptors and primer sequences, the raw sequnences were assembled for each sample according to the unique barcode using the QIIME pipeline (Quantitative Insights Into Microbial Ecology, v 1.8.0, qiime.org) (Caporaso et al. 2010). The low-quality sequences were filtered through following criteria (Gill et al. 2006; Chen and Jiang 2014): sequences that had a length of <150 bp, sequences that had average Phred scores of <20, sequences that contained ambiguous bases, and sequences that contained mononucleotide repeats of >8 bp. Paired-end reads were assembled using FLASH (v1.2.7) (Magoč and Salzberg 2011). After chimera detection, the remaining high-quality sequences were clustered into operational taxonomic units (OTUs) at 97% sequence identity by UCLUST (Edgar 2010 ). A representative sequence was selected from each OTU using default parameters. OTU taxonomic classification was conducted by BLAST searching the representative sequences set against the Greengenes Database (DeSantis et al. 2006) using the best hit (Altschul et al. 1997) and the fungal sequences were matched against the UNITE database (Release 5.0, https://unite.ut.ee/) (Kõljalg et al. 2013). An OTU table was further generated to record the abundance of each OTU in each sample and the taxonomy of these OTUs. OTUs containing less than 0.001% of total sequences across all samples were discarded. To minimize the difference of sequencing depth across samples, an averaged, rounded rarefied OTU table was generated by averaging 100 evenly resampled OTU subsets under the 90% of the minimum sequencing depth for further analysis.

## Bioinformatics analysis

Sequence data analyses were mainly performed using QIIME (v1.8.0) and R packages (v3.2.0). OTU-level alpha diversity indices, such as Chao1 richness estimator, ACE metric (Abundance-based Coverage Estimator), Shannon diversity index, and Simpson index, were calculated using the OTU table in QIIME (v1.8.0). OTU-level ranked abundance curves were generated to compare the richness and evenness of OTUs among samples (Caporaso et al. 2010). To explore variation in fungal and bacterial community structures across the soil samples analyzed, weighted UniFrac distance was also performed in Mothur (v.1.25.1). PCoA (Principal Coordinate Analysis) was performed on distance matrices, and coordinates were used to draw 2D graphical outputs (Lozupone et al. 2007). The linear discriminant analysis (LDA) effect size (LEfSe) method was used to detect differentially abundant taxa across groups using the default parameters through the Galaxy online analytics platform (http://huttenhower.sph.harvard.edu/galaxy/) (Segata et al. 2011). An alpha value of 0.05 was used for the factorial Kruskal-Wallis test, and the threshold was set at 3.0 for the logarithmic LDA score for a discriminative feature. Hierarchical clusters were generated in Mothur (v.1.25.1) using cluster and heat map was drawn for each each sample in terms of abundance using R packages (v3.2.0). Co-occurrence analysis was performed by calculating Spearman's rank

correlations between dominant genera of the top 50 abundances.

Correlations with |RHO| > 0.6 and $P < 0.01$ were visualized as co-occurrence network using Cytoscape software (www.cytoscape.org) (Shannon et al. 2003).

### Statistical analysis

Soil physicochemical characteristics were compared using Student's t-test ($P < 0.05$). The number of OTUs, alpha diversity indices, and the taxa (phyla and genus) bacterial and fungal relative abundances were calculated for all replicates and subjected to an analysis of variance by one-way ANOVA ($P < 0.05$). These analyses were performed in SPSS version 16.0 (SPSS Inc., Chicago, IL, USA).

### Data accession numbers

All raw sequences were deposited in the NCBI Sequence Read Archive (SRA) database under the accession number SRP163101.

## Results

### The effect of cultivation of American ginseng on soil physiochemical properties

Soil characteristics for the two types of fields are summarized in Table 1. Compared with the uncultivated soil (Control), the soil from farmland used to cultivate American ginseng for 4 years (CAG) had a significantly ($P < 0.05$, Student's t-test) higher pH, electrical conductivity (EC), organic matter (OM) content, and available P. The available N and K values showed no significant ($P < 0.05$, Student's t-test) difference between the control and CAG soils.

### Analysis of amplicon-data and microbia community diversity

High quality sequences of 381, 992 reads for 16S rRNA genes and 303, 581 reads for ITS genes were obtained for an average of 42, 444 sequences of bacterial 16S rRNA genes (most common length ranged from 420 460 bp) and 33, 731 fungal ITS reads (most common length range 200 320 bp) (**Table S1 and Fig. S2**). A total of 25, 918 OTUs of bacterial phyla and 3, 907 OTUs of fungal phyla from the 9 samples were obtained, and 17, 405 OTUs of bacterial phyla and 3, 175 OTUs of fungal phyla were obtained after discarding OTUs less than 0.001% of total sequences across all samples (**Table S1**). Although there were nearly ten thousand sequences per soil sample representing the bacterial community, the slope of the rarefaction curve did not reach linearity at different similarity cutoff values, indicating that there were bacteria that were not detected (**Fig. S3a**). Conversely, the slope of the rarefaction curve for the fungal species was flat at different similarity cutoff values, indicating that the identified fungal diversity was close to saturation and that an increase in the sequencing depth would not help to observe more fungal species **(Fig. S3b**).

Cultivation of American ginseng significantly increased the number of bacterial OTUs in the soil, but decreased the number of fungal OTUs (Table 2). For the bacterial community, the Simpson and Shannon indices showed no significant changes (ANOVA, $P < 0.05$), but when the number of OTUs were calculated by Chao1 and ACE richness estimators, the numbers increased significantly (ANOVA, $P < 0.05$) in the soil culturing American ginseng compared to the control soil samples. This indicated that culture of American ginseng resulted in an increase in the richness of the bacterial community. The Simpson, Chao1, ACE and Shannon values for the fungal community all significantly decreased (ANOVA, $P < 0.05$) in the soil used for cultivating American ginseng for 4 years compared with the control soil

**Table 1** Summary of soil characteristics from an uncultivated plot (Control) and a field growing American ginseng for 4 years (CAG)

| Samples | pH | EC (ms/cm) | OM (g/kg) | Available N (mg/kg) | Available P (mg/kg) | Available K (mg/kg) |
|---|---|---|---|---|---|---|
| Control | 5.53±0.02b | 0.50±0.05b | 13.74±0.95b | 31.33±8.64a | 7.97±0.55b | 282.89±118.72a |
| CAG | 6.26±0.23a | 1.15±0.53a | 50.17±16.33a | 35.29±4.32a | 29.10±9.48a | 348.13±199.86a |

Each value represents the mean ± standard deviation (n = 3). Means followed by a different letter for a given factor are significantly different ($P < 0.05$; Student's t-test)

**Table 2** Summary of the bacterial and fungal diversity and richness indices of the control soil (C) and the rhizosphere soils of healthy ginseng (H) and root rot-diseased ginseng (R)

| Sample names | Bacteria | | | | | Fungi | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | OTU Numbers | Simpson | Chao1 | ACE | Shannon | OTU Numbers | Simpson | Chao1 | ACE | Shannon |
| C | 260.20±1.83b | 0.60±0.00a | 1, 943.43±162.89b | 1, 977.95±220.93b | 6.08±0.01a | 293.00±17.23a | 0.57±0.01a | 566.03±36.90a | 567.91±37.24a | 3.84±0.19a |
| H | 387.20±8.43a | 0.59±0.00a | 2, 241.40±86.49a | 2, 319.06±27.63a | 5.77±0.02a | 182.40±9.43b | 0.47±0.01b | 349.20±17.15b | 349.20±17.15b | 2.62±0.04b |
| R | 358.6±33.95a | 0.60±0.01a | 2, 267.13±390.94a | 2, 329.94±456.13a | 5.93±0.28a | 144.60±20.07c | 0.50±0.03b | 284.95±35.32c | 286.91±37.02c | 2.50±0.31b |

The value of each bar represents the mean ± standard deviation (n = 3). Means followed by a different letter for a given factor are significantly different between samples in the same column according to Duncan's mean test ($P < 0.05$; one-way ANOVA)

sample, indicating that culture of American ginseng caused a decline in diversity and richness of the fungal community. Additionally, the diversity and richness indices showed no significant changes (ANOVA, $P < 0.05$) in the bacterial communities between the rhizosphere soils of healthy and diseased roots, whereas the richness indices (Chao1 and ACE) in the fungal community showed a significant decrease in rhizosphere soils of root rot diseased ginseng than that of healthy ginseng.

## Microbial community similarities in different samples

The similarity of the microbial communities in the soil samples of C, H and R was determined by weighted-UniFrac principal coordinates analysis (PCoA; Fig. 2). As shown in Fig. 2, the weighted-UniFrac PCoA showed that both bacterial and fungal communities from the soil of C, H and R were clearly separated from each other (Fig. 2), and the difference was statistically significant (ANOSIM, R = 0.967, P = 0.006 for bacteria; R = 0.893, P = 0.001 for fungi). The principal coordinates PC1 and PC2 represented 65.69% and 21.33%, respectively, of the variance, and the contribution of the cumulative variance of the two principal coordinates (PC1 and PC2) accounted for 87.02%. As for the fungal communities, PC1 and PC2 represented 74.47% and 14.70%, respectively, of the variance, and the contribution of the cumulative variance of the two principal coordinates (PC1 and PC2) accounted for 89.17%. This was also demonstrated by hierarchical trees constructed through weighted pair-group method using arithmetic averages (WPGMA) calculations. This clustering showed that the bacterial and fungal communities in the rhizosphere soil of both healthy and diseased ginseng appeared more similar to each other than to those in the control, indicating that culture of American ginseng remarkably changes the microbial community in the soil (**Fig. S4**).

## Microbial community composition and structure

The microbial populations in the different samples were analyzed by submitting the obtained 16S rRNA and ITS sequences to a local blast search against the Greengenes Database and UNITE databases (Fig. 3). The dominant bacterial phyla were *Proteobacteria, Actinobacteria, Chloroflexi, Acidobacteria, Gemmatimonadetes* and *Nitrospirae* across the three sample groups (Fig. 3a).

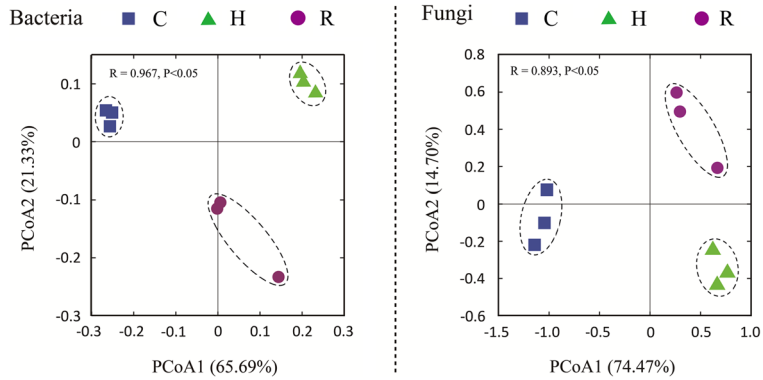


**Fig. 2** UniFrac-weighted principle coordinate analysis (PCoA) of bacterial and fungal community structures in the control soil (C) and the rhizosphere soils of healthy (H) and root rot-diseased (R) ginseng. Analysis of similarities (ANOSIM) was performed to evaluate the significant differences in microbial communities using QIIME (v1.8.0) ($P < 0.05$)

*Proteobacteria* was predominant in each of the samples, at 31.41, 36.39 and 36.44% of the total bacterial phyla detected in the C, H and R samples, respectively (Fig. 3a). The most populous bacterial genera were also the same across the 3 samples, namely *Kaistobacter*, *Rhodoplanes*, *Sphingobium*, *Arthrobacter, Blastococcus, Candidatus solibacter, Nitrospira*, *Mycobacterium, Phenylobacterium* and *Bacillus* (Fig. 3b). The proportions of *Nitrospira* (1.78%) and *Kaistobacter* (1.55%) were highest in the control sample (C). In the healthy ginseng soil samples (H), the proportions of *Rhodoplanes* (5.51%) and *Kaistobacter* (3.78%) were more abundant than other bacteria. And the proportions of *Sphingobium* (4.01%) and *Rhodoplanes* (1.46%) in the rhizosphere soil of rotten plants (R) were predominant.

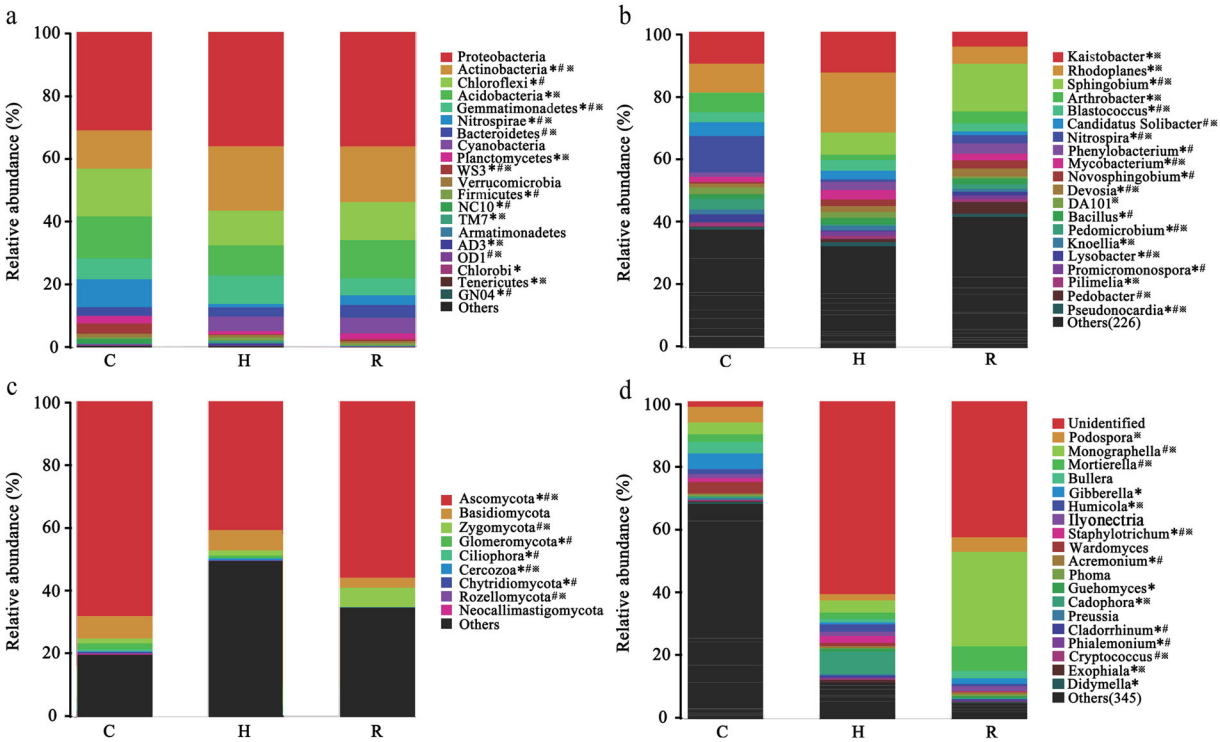


**Fig. 3** The composition and structure of the bacterial and fungal community from the control soil sample (C) and the rhizosphere soil samples from healthy ginseng (H) and root rot-diseased ginseng (R). Bacterial community at the phylum level (**a**) or the genus level (**b**); Fungal community at the phylum level (**c**) or the genus level (**d**). The phyla with relative abundances in the top 20 were chosen to exhibit. Others represents phyla of low relative abundance that ranks lower than 20. *, # and  represent significant differences ($P < 0.05$) according to one-way ANOVA (n=3). *, # and  stands for C:H, C:R and H:R, respectively

For the fungal community, we detected three main phyla in the samples: *Ascomycota*, *Basidiomycota* and *Zygomycota* (Fig. 3c). *Ascomycota* was the most dominant group in all three soil samples at the phylum level, representing 68.06%, 40.77% and 55.90% in the C, H and R groups, respectively (Fig. 3c). The limited resolution of ITS sequencing meant that there was a notable fraction of the ITS sequences in each sample that could not be exactly classified, especially in the rhizosphere samples, with 46.07 (H) and 33.25% (R) of the sequences undefined in these two samples, respectively (Fig. 3c). Similarly, undefined sequences accounted for a very high proportion in the soil samples at the genus level (Fig. 3d). The most abundant genera detected in the control soil were *Podospora*, *Gibberella* and *Schizothecium*, at 2.52% of the total sequences, while other genera, such as *Devriesia* (2.27%), *Monographella* (1.91%) and *Bullera* (1.88%) were also detected. The main genera detected in the rhizosphere soil of healthy ginseng were *Cadophora* (5.32%) and *Monographella* (2.90%) except for unidentified genera. In the root rot ginseng sample, *Monographella* (23.13%) and *Mortierella* (6.00%) were the main genera detected.

In order to the further analysis a significant difference of relative abundances, we performed a significance analysis for the relative abundance of the microflora using one-way ANOVA, and the results had been marked in the Fig. 3 with different symbols. In addition, a heat map was also used to intuitively show the differences of the relative abundances of bacterial and fungal genera observed, and to highlight the particularly high or low genus in each sample using a colored frame (**Fig. S5**). The relative abundances with the top 50 of the highest contribution to the ordination were selected.

### The LEfSe analysis of the differentially abundant feature from bacteria and fungal communities

LEfSe uses LDA scores to estimate the effect size of each differentially abundant feature, and to rank the relative difference of microbial taxa that are discriminative with biological consistency and statistical significance. The LEfSe analysis of the bacterial communities from C, H and R soil samples shows that there are 47 differentially abundant taxonomic clades with a LDA score higher than 3.0 (Fig. 4). Out of the 47 different bacterial genera, 21 genera were differentially abundant in the control soil sample (C) (LDA score > 3.0), including *Nitrospira*, *Janthinobacterium*, *Arthrobacter*, *Candidatus_Solibacter*, *Pedomicrobium*, *Flavobacterium* and so on. The most differentially abundant bacterial taxa in the H soil sample belong to genera: *Rhodoplanes* and *Kastobacter* (LDA score > 4.0). The differential genera overrepresented in the rhizosphere soils of R samples include *Sphingobium*, *Pedobacter*, *Pseudomonas* and *Sphingopyxis* (LDA score > 4.0).

The LEfSe analysis of the fungal communities from C, H and R samples shows that there are 28 differentially abundant taxonomic clades with a LDA score higher than 3.0 (Fig. 4). Out of the 28 different fungal genera, 16 genera were differentially abundant in the control soil samples (C) (LDA score > 3.0), including *Mrakia*, *Gibberella*, *Lasiosphaeriaceae*, *Devriesia*, *Racocetra*, *Acicuseptoria*, *Pyrenochaetopsis*, *Myrothecium* and so on. The genera overrepresented in the rhizosphere soils of H samples include *Melanophyllum* and *Staphylotrichum* (LDA score > 3.5). The most differentially abundant fungal taxa in the rhizosphere soils of R sample belong to genera: *Mortierella* (LDA score > 4.0).

In order to further exhibit the possible "collaborative" or "competing" relationships among different communities, Spearman's rank correlation coefficients between the most abundant genera were calculated using the Mothur software. The correlations among the 49 dominant bacterial genera and the 22 fungal genera were analyzed (**Fig. S6**).

## Discussion

Soil pH was a major factor in defining microbial diversity through impacting pH homeostasis of microbial cell or regulating availability of soil nutrients (Fierer and Jackson 2006; Rousk et al. 2010). In this study, cultivation of American ginseng for 4 years significantly improved ($P < 0.05$; Student's t-test) the soil pH compared with the control (Table 1). This is also in line with a report of Tan et al. (2017), and their results showed that the soil pH significantly increased after 3 years of cultivation of *Panax notoginseng* (Tan et al. 2017). Zhalnina et al. (2015) reported that pH is the main driver of microbial community in the park grass soil and pH changes in soils are the result of nutrient management, and these pH changes affect nutrient availability to both plant and microbial communities. Agricultural soils are commonly fertilized with nitrogen, and this could be

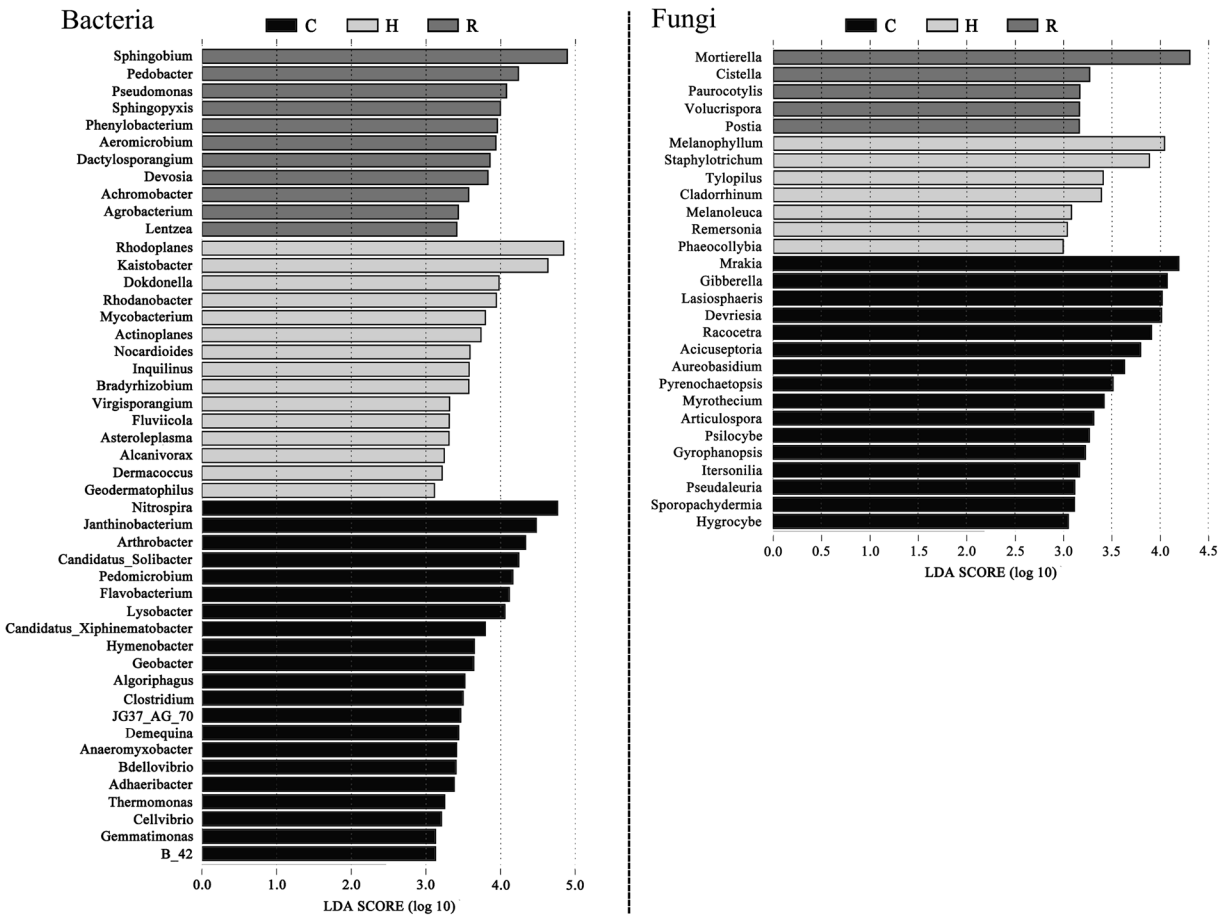


**Fig. 4** Differentially abundant bacterial and fungal taxa as assessed using linear discriminant analysis (LDA) with effect size measurements (LEfSe) in the control soil (C) and the rhizosphere soils of healthy (H) and root rot-diseased (R) ginseng. LDA score > 3.0 and $P < 0.05$

another possible factor regulating the diversity of microorganisms (Zhalnina et al. 2015). However, there are no significant difference ($P < 0.05$; Student's t-test) between the control and CAG sample soil. Additionally, the CAG soil had a significantly ($P < 0.05$; Student's t-test) higher EC, OM and available P content compared to the control soil. Whether these changes (including EC, OM and available P) can affect the soil microbiota community still need to further study.

Soil microorganisms have profound effects on the growth, nutrition and health of plants in natural and agricultural ecosystems (Garbeva et al. 2004; Almario et al. 2013). Characterizing the diversity and richness of the microbial communities in the rhizosphere soils of healthy ginseng and root rot ginseng is an important first step toward understanding whether these communities influence the occurrence of root rot diseases. In this study, soil growing American ginseng for 4 years harbored a significantly (ANOVA, $P < 0.05$) higher bacterial richness and lower fungal richness and diversity than the control soil (Table 2). In contrast, Dong et al. (2017) showed that less diverse bacterial communities and more diverse fungal communities were found in the rhizosphere soil of American ginseng compared to the rhizosphere soil of maize. Compared with the rhizosphere soil of a healthy ginseng root (H), the soil of diseased ginseng (R) showed a significant decrease (ANOVA, $P < 0.05$) in the fungal richness (Table 2). This result is in line with a study in vanilla showing that soil conducive to *Fusarium* wilt disease harbored a significantly lower fungal abundance and diversity than the suppressive soil (Xiong et al. 2017). Tan et al. (2017) also reported that fungal diversity in the root-rot diseased notoginseng rhizospheric soil decreased, in contrast, fungal diversity in healthy notoginseng rhizospheric soil increased with increasing years of

continuous cropping years. On the other hand, soil microbial communities are also influenced by multiple factors such as plant type, climate, soil properties and agricultural practice (Burton et al. 2010; Hao et al. 2009).

Changes in the composition of the bacterial community may lead to variations in metabolic capacity, biodegradation, and disease-suppression abilities (Garbeva et al. 2004; Bell et al. 2013). Analysis of the bacterial proportions showed that *Proteobacteria* and *Actinobacteria* were the most high at phyla level in the three group samples (Fig. 3a). *Actinobacteria*, producing a large and diverse array of bioactive compounds that inhibit the development of pathogens in the soil, are some of the microorganisms with a role of biological control that have been extensively studied (Sharma et al. 2005). And at the genus level, *Nitrospira* (1.78%), *Rhodoplanes* (5.51%) and *Sphingobium* (4.01%) was predominant in the C, H and R sample, respectively (Fig. 3b). As for fungi, *Ascomycota*, *Basidiomycota* and *Zygomycota* were the most abundant fungal phyla identified in the different samples (Fig. 3c). Li et al. (2014) reported that *Ascomycota* and *Basidiomycota* were the top two prevalent fungal phyla in a continuous cropping peanut system. Tan et al. (2017) also reported that *Ascomycota*, *Basidiomycota* and *Zygomycota* were dominant phyla during the continuous cropping of *P. notoginseng*. This result is also in agreement with a previous study in which *Ascomycota* and *Basidiomycota* were the most abundant fungal phyla identified in soil conducive to *Fusarium* wilt disease (Xiong et al. 2017). *Ascomycetes* occur in terrestrial, marine, and freshwater habitats, and many species play a major ecological role as decomposers (Miadlikowska et al. 2006). Notably, *Monographella* was significantly higher in the rhizosphere soil of diseased ginseng than healthy ginseng (Fig. 3d). It is reported that *Monographella* is a fungal plant pathogen and contains many plant pathogenic species (Aveskamp et al. 2010).

The composition of the bacterial community analysis showed that *Nitrospira*, *Janthinobacterium* and *Pedomicrobium* were significantly (ANOVA, $P < 0.05$) decreased after culture of American ginseng (Fig. 4). *Nitrospira* and *Pedomicrobium* are ubiquitous bacteria that function in the nitrogen cycle and take advantage of the nutrients, respectively. *Nitrospira* has been shown to perform nitrite oxidation in the second step of nitrification (Koch et al. 2015). Larsen et al. (1999) showed that *Pedomicrobium* is able to greatly enhance the rate of Mn oxidation. It is reported that *Janthinobacterium,* a genus of gram-negative soil bacteria, can produce a purple-violet pigment, manifest diverse energy metabolism abilities, and tolerate cold, ultraviolet radiation, and other environmental stressors (Koo et al. 2016). With respect to fungi, *Paradendryphiella*, *Psilocybe*, *Sporopachydermia, Myrothecium*, *Racocetra*, *Aureobasidium* and *Coprinellus* were remarkable decreased after culture of American ginseng. *Psilocybe* and *Coprinellus* are saprotrophic species, deriving nutrients from dead and decomposing organic matter, and grow in and around stumps or logs of broad-leaved trees or attached to buried wood (Borovička et al. 2014; Redhead et al. 2001). Other newly discovered genera such as *Sporopachydermia, Paradendryphiella*, *Myrothecium, Aureobasidium* and *Racocetra* were also significantly (ANOVA, $P < 0.05$) decreased after culture of American ginseng, but their potential functions are not yet clear.

The LEfSe analyses revealed that there were indeed differences in the relative abundances of both bacterial and fungal genera between the soils accompanying healthy and root rot-diseased ginseng roots. *Rhodoplanes, Kaistobacter, Dokdonella* and *Actinoplanes* bacteria were more differently abundant in the rhizosphere soils of healthy ginseng, whereas the *Pedobacter*, *Pseudomonas*, *Sphingopyxis* and *Dactylosporangium* bacteria were more differently abundant in the rhizosphere soils of root rot ginseng (Fig. 4). *Rhodoplanes* and *Kaistobacter* were identified as indigenous degraders for atrazine and useful for atrazine bioremediation (Lodha et al. 2015; Wei et al. 2018). *Dokdonella* is related to pyridine biodegradation and could be a potential alternative for the enhancement of pyridine removal from waste water in anaerobic systems (Liu et al. 2013). *Actinoplanes*, a genus in the family *Micromonosporaceae*, is able to produce the pharmaceutically important compounds valienamine (a precursor to the anti-diabetic drug acarbose and the antibiotic validamycin), teicoplanin and ramoplanin (Laube 2002). In spite of these bacteria might exhibit the feature of degrading harmful compounds in the soil, such as atrazine and pyridine (Liu et al. 2013; Lodha et al. 2015; Wei et al. 2018), whether the species associated with root-rot disease of American ginseng still needs further investigations. Surprisingly, the *Pseudomonas* bacteria is rich in the rhizosphere soils of diseased ginseng roots. Because many *Pseudomonas* produce the antifungal compounds 2,4-diacetylphloroglucinol (Phl) and/or hydrogen cyanide (HCN) and were antagonistic to

*T. basicola* in vitro and to protect tobacco from black root rot (Stutz et al. 1986; Ramette et al. 2003).

*Mortierella* genus was the most dominant fungal genus in the rhizosphere soils of root rot-diseased American ginseng (Fig. 4). A culture-dependent study showed that *Phoma* was the main fungal genera observed as obstacles in continuous cropping (Miao et al. 2006). Some studies have shown that some species of *Mortierella* can produce antibiotics, and several isolates have been investigated as potential antagonistic agents against various plant pathogens (Tagawa et al. 2010; Wills and Lambe 1980). Additionally, *Melanophyllum,* a genus of fungi in the family *Agaricaceae*, is a hyper-dominant in the rhizosphere soils of healthy ginseng. Although *Fusarium* spp., *Phytophthora* spp. and *Cylindrocarpon* spp. were often reported to cause severe root rot and could be frequently isolated from cultivated American ginseng root (Reeleder and Brammall 1994; Punja 1997; Punja et al. 2007; Bi et al. 2011), these fungi were not detected from the rhizosphere soils of the diseased ginseng in this study. The research results of Agler et al. (2016) dementated that not all pathogens share the hub microbe or even keystone status, so pathogenicity cannot be taken as a rule to detect "hub" or "keystone" species. The microbia diversity manipulation might be a key battleground where hosts and various hubs cooperate or compete with one another (Agler et al. 2016). *Phoma* is a genus of common coelomycetous soil fungi and contains many plant pathogenic species (Aveskamp et al. 2010). For example, *Phoma beta* is the cause of the heart rot and blight of beets and *Phoma batata* can cause a dry rot of sweet potato. Conversely, the fungi that secrete antibiotics, such as *Trichoderma* and *Penicillium*, were relatively higher in the rhizosphere soil of healthy American ginseng compared with that of diseased American ginseng.

In addition, microbe-microbe interactions generally increase host effects due to the community correlation network topology (Agler et al. 2016). In this study, a map of the microbial networks was also provided to exhibit a possible "collaborative" or "competing" correction within the microbial communities in the rhizosphere soils of American ginseng (**Fig. S6**). For example, the bacterial genera *Pedomicrobium*, *Janthinobacterium*, *Nitrospira*, *Lysobacter*, *Candidatus*. *Xiphinematobacter* and *Flavobacterium* might kept a "collaborative" relationship with each other in bacterial community. These relationships between bacteria and fungi might provide some clues for the further screening of bacteria antagonistic to root rot disease in American ginseng.

## Conclusions

In this study, our results indicated that the microbial community differs between the soil attached to healthy and rotted roots of American ginseng, and some hyper-dominant bacteria and fungi were also founded in the rhizosphere soils of health and rotten root. However, soil microbial communities are influenced by multiple factors such as plant type, climate, soil properties and agricultural practice (Burton et al. 2010; Hao et al. 2009). In addition, complex interactions take place between microorganisms and roots, and the effective plant-protecting populations may be influenced by accompanying microbiota (Duijff et al. 1999; Raaijmakers et al. 2008). Therefore, further studies identifying the hyper-dominant taxon isolates with effective rotten root disease suppression ability and revealing their interactions may open new avenues for the development of informed bio-control strategies.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare no conflicts of interest.



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