

## Effects of ginseng cultivation on rhizosphere soil microecological environment

C.X. Yang, S.H. Luo<sup>1</sup>, J. Wang<sup>2</sup>, J.J. Zhu<sup>2</sup>, H.L. Chen<sup>2</sup>, Y.F. Zhou<sup>2</sup> and X.S. Zhao\*

Liaoning Technical University, Fuxin 123000, China

E. Mail: zhaoxs210@163.com

(Received in revised form: September 7, 2021)

### ABSTRACT

This research aimed to determine the allelopathic effects of *Panax ginseng* C. A. Meyer mediated via ginsenosides on rhizosphere soil microbial community. We analyzed using high-throughput sequencing technology, the soil microbial community structure and diversity of forest soil and ginseng rhizosphere soils cultivated with *P. ginseng* for 4, 5 and 6 years. In soil samples, the contents of ginsenoside monomers Rb<sub>1</sub>, Rb<sub>2</sub>, Rc, Rd, Rg<sub>1</sub>, Re, G-VXII and F<sub>2</sub> were determined using UPLC-MS/MS. The results showed that compared with forest soil control, the community structures of fungi and bacteria significantly increased in ginseng rhizosphere soils, and the fungal community's diversity clearly changed in ginseng rhizosphere soil. The relative abundance of ginseng pathogenic fungi (*Fusarium* and *Ilyonectria*) in rhizosphere soils significantly increased after ginseng was planted. During the ginseng growth, the relative contents of ginsenosides (Rb<sub>1</sub>, Rb<sub>2</sub> and Rc) secreted through ginseng roots in the rhizosphere soils decreased, while, the relative contents of ginsenosides degradation products (Rd, G-VXII and F<sub>2</sub>) increased. Unifrac PCoA analysis showed significant differences in the soil microbial community structure across various ginseng cultivation years. RDA analysis showed that ginsenosides were key factors affecting the soil microbial community structure and diversity.

**Key Words:** Allelochemicals, bacteria, continuous cropping obstacles, forest soil, fungi, ginsenoside, high-throughput sequencing, *Panax ginseng*, soil microbial community

### INTRODUCTION

*Panax ginseng* C. A. Meyer is a rare medicinal plant with important medicinal value and economic benefits. The main producing area of *P. ginseng* is mainly dependent on artificial cultivation in China. Ginseng plants and its mature roots are shown in Photograph 1. Ginseng is harvested 4-6 years after sowing and subsequent replanting commonly fails due to continuous cropping problems, which has become a major problem that needs to be solved urgently. Multiple factors (outbreak of soil-borne diseases, deterioration of soil physico-chemical properties, allelopathy and changes in soil microbial community composition) are implicated in continuous cropping problems of *P. ginseng* (1).

Ginsenosides are the most important secondary metabolites produced by ginseng. Nicol *et al.* (9,10) found that American ginseng (*Panax quinquefolius* L.) released ginsenosides into the rhizosphere through the roots during the growth. Ginsenosides promote the pathogen's growth (*Phytophthora cactorum* and *Pythium irregular*) and inhibits the biocontrol fungus *Trichoderma hamatum* under the same conditions.

---

\*Correspondence author, <sup>1</sup>Shenyang Agricultural University, Shenyang 110000, China, <sup>2</sup>Northeast Normal University, Changchun 130000, China.



Photograph 1. Ginseng plants and its mature roots

*P. irregulare* produces glycoside hydrolase that degrades the ginsenosides. This enzyme removes the glucose or arabinose from the sugar chain ends of protopanaxadiol (PPD)-type ginsenosides to produce compound F<sub>2</sub> (15). Ivanov *et al.* (4) found that ginsenosides have chemotactic effects, which attracted the *P. irregulare* to accumulate in the ginsenosides rich rhizosphere. The chemo-attractant activity of ginsenosides interacts with ginseng pathogens [*Fusarium solani*, *Cylindrocarpon destructans*, *Rhizoctonia solani* and *Sclerotinia schinseng*] (12,16). Our group studied the interactions between the ginsenosides extracted from the roots of *P. ginseng* and soil fungi showed that total ginsenosides, PPD-type ginsenosides and ginsenoside monomers Rb<sub>1</sub> and Rb<sub>2</sub> promoted the growth of *C. destructans*, while, inhibiting the growth of non-ginseng pathogens (13,19). *C. destructans* produces extracellular glycosidase that hydrolyzes the PPD-type ginsenosides, the specificity of this enzyme to hydrolyze PPD-type ginsenosides is similar to that of glycosidase secreted by *P. irregulare*. In addition, several non-ginseng pathogens also degrade the ginsenosides and the degradation path is similar to *C. destructans* (13,19). These results indicated that ginsenosides will affect the composition and diversity of the microbial community after entering the rhizosphere soil, while soil fungi will metabolize ginsenosides. Thus, the interactions between plants and fungi pathogens mediated via ginsenosides are key factors resulting in microflora imbalances in ginseng soil.

In this study, we collected the ginseng rhizosphere soil samples with different planting years and analyzed the soil bacterial and fungal communities for structure and diversity by Illumina PE250 high-throughput sequencing. We also determined the contents of 6-main ginsenosides (Rb<sub>1</sub>, Rb<sub>2</sub>, Rc, Rd, Re, Rg<sub>1</sub>) and two ginsenoside degradation products (GXVII, F<sub>2</sub>) in the soil samples using the UPLC-MS/MS. This study aimed to determine the allelopathic effects of *Panax ginseng* C. A. Meyer mediated via ginsenosides

on rhizosphere soil microbial community. These findings may help to understand the replanting problem and to develop strategies to overcome it.

## MATERIALS AND METHODS

### I. Study site

Study area is a farmland located in Yangquan town, Fusong county, Jilin Province, China (127°17'N, 42°26'E, 744 m), the main region for ginseng production. It has a continental climate, mean annual rainfall: 830 mm, annual mean temperature: 4.0 °C, mean annual sunshine: 2352.5 h. The soil is Chernozem.

Table 1. Physico-chemical properties of soil samples

Samples	pH	Organic matter(g·kg <sup>-1</sup> )	Total N (g·kg <sup>-1</sup> )	Available N (mg·kg <sup>-1</sup> )	Total P (g·kg <sup>-1</sup> )	Available P (mg·kg <sup>-1</sup> )	Total K (g·kg <sup>-1</sup> )	Available K (mg·kg <sup>-1</sup> )
CT	5.25±0.02 <sup>b</sup>	10.31±0.04 <sup>a</sup>	4.78±0.23 <sup>a</sup>	126.33±0.76 <sup>b</sup>	0.98±0.00 <sup>a</sup>	22.22±0.04 <sup>a</sup>	73.49±0.11 <sup>b</sup>	84.33±0.42 <sup>b</sup>
GS2+2	5.71±0.02 <sup>a</sup>	7.01±0.08 <sup>c</sup>	1.81±0.05 <sup>b</sup>	136.17±0.97 <sup>a</sup>	0.94±0.00 <sup>b</sup>	13.22±0.00 <sup>b</sup>	73.2±0.26 <sup>b</sup>	92.60±1.26 <sup>a</sup>
GS2+3	4.06±0.01 <sup>d</sup>	5.36±0.11 <sup>d</sup>	0.96±0.07 <sup>d</sup>	117.43±0.54 <sup>c</sup>	0.10±0.00 <sup>c</sup>	4.56±0.00 <sup>d</sup>	81.85±0.21 <sup>a</sup>	76.54±1.57 <sup>d</sup>
GS2+4	4.47±0.01 <sup>c</sup>	9.31±0.16 <sup>b</sup>	1.47±0.05 <sup>c</sup>	112.1±1.47 <sup>d</sup>	0.01±0.00 <sup>d</sup>	10.87±0.00 <sup>c</sup>	66.48±0.07 <sup>c</sup>	80.43±1.02 <sup>c</sup>

GS 2: Ginseng seeds were sown in forest soil and after 2 years, its seedlings were transplanted into new forest soils and cultivated for 2, 3, 4 years until harvest. This is the main cultivation pattern in ginseng-producing regions. CT : Forest soils, GS2+2 : Ginseng soil after 2+2, GS2+3 : Ginseng soil after 2+3 and GS2+4 : Ginseng soil after 2+4 represent the ginseng rhizosphere soil after 2+2, 2+3 and 2+4 years, respectively. Data are presented as means ± SEs (n = 3). Different lowercase letters indicate significant differences between soil samples (P<0.05).

Ginseng is cultivated according to regional practices and followed the standard operating procedures of Good Agricultural Practice (3,17). The farmland was covered with forest soil from 35-year old forest, before ginseng planting. Two-year old ginseng seedlings grown in this soil were transplanted into new forest soils and cultivated for 2, 3, 4 years until harvest. This is the main cultivation pattern in ginseng-producing regions. The Ginseng plantation comprised of 4-plots, each plot contained 3-Ginseng-beds (20 m long, 1.5 m wide and 30 cm high) (Photograph 2). Ginseng seedlings after transplanting were grown for 2, 3, or 4 years. The soil samples from ginseng-cultivated land were referred as GS 2+2, GS2+3 and GS 2+4, respectively. Where GS 2: Ginseng seeds were sown in forest soil and after 2 years, its seedlings were transplanted into new forest soils and cultivated for 2, 3, 4 years until harvest. This is the main cultivation pattern in ginseng-producing regions. A forest soil (CT) plot without cultivation of ginseng served as control. CT : Forest soils, GS2+2 : Ginseng soil after 2+2, GS2+3 : Ginseng soil after 2+3 and GS2+4 : Ginseng soil after 2+4 represent the ginseng rhizosphere soil after 2+2, 2+3 and 2+4 years, respectively. As per S-shaped sampling method, control soil samples from 5-sites (5 cm dia and 20 cm depth) were collected by auger and combined and homogenized into one sample. Five ginseng seedlings were randomly selected from each Ginseng-bed. Rhizosphere soil adhering to ginseng roots was collected by brushing the roots and soil samples from each Ginseng-bed were pooled together as one sample. Twelve samples were collected, homogenized sieved through a 2 mm sieve and divided into 2-sub-samples. A sub-sample was used to determine the physico-chemical properties of soil and the other was stored at -80 °C for further use.



Photograph 2. Soil bed for Ginseng sowing

## II. Soil physico-chemical properties

Physico-chemical properties of soil samples were determined as per (7) (Table 1). Ginsenosides from soil extracts were determined by ultra-performance liquid chromatography-tandem mass spectrometry [UPLC-MS/MS, Shimadzu International Trading (Shanghai) Co., Ltd.; Shim-pack GIST C<sub>18</sub>, equipped with a 100 mm×2.1 mm×2 μm chromatographic column]. Each soil sample was weighed 3.0 g and placed in a conical flask, methanol was added twice for ultrasonic extraction. The supernatant of two extracts was combined and concentrated by rotary evaporation. Chromatographic methanol was added after solvent removal and filtered through 0.22 mm filter membrane to a constant volume of 1.0 mL, 0.1 %. Formic acid was used as mobile phase A and acetonitrile was used as mobile phase B. The flow rate was 0.2 mL·min<sup>-1</sup>. The column temperature was 35 °C. The injection volume was 10 μL. The gradient elution method was used and the gradient elution conditions were 0-12 min, 5-95 % B, 12-13 min, 95 % B. The compound was detected in negative ion mode by MRM mode. The interface temperature was 300 °C, DL temperature was 250 °C, heating block temperature was 450 °C. The drying gas flow rate was 15 L·min<sup>-1</sup> and the heating gas flow rate was 5 L·min<sup>-1</sup>.

## III. Soil bacterial and fungal community analysis

All soil DNA extraction and sequencing were done in Shanghai Ling 'en Biotechnology Co., Ltd. Using primers pair 341F: CCTACGGGRBGCASCAG and 806R: GGACTACNNGGGTATCTAAT to amplify the bacterial V3-V4 region of 16S rRNA gene by PCR. Using primers pair ITS1F: 5'-TCGCTACTACCGATTGGATGGTTTA-3' and ITS2R: 5'-GCTCTTCCCTCTTCACTCG-3' to amplify the fungal ITS1 region by PCR. The data were analyzed by Biomark technology cloud platform.

(i). **Microbial community structure analysis:** Sequence analysis were performed using QIIME platform ([http://qiime.org/scripts/assign\\_taxonomy.html](http://qiime.org/scripts/assign_taxonomy.html)). Operational

taxonomic units (OTUs) were clustered with 97 % similarity. Research software (version 10, <http://drive5.com/uparse/>) was used to remove single sequences without repetition and chimeras were removed in the clustering process to obtain the representative sequence of OTU. The RDP Classifier Bayesian algorithm was used for the taxonomic analysis of 97 % OTU representative sequences with similar levels and community composition of each sample was counted at phylum and genus classification levels.

**(ii).  $\alpha$ -diversity analysis:** Chao1 index was calculated to estimate the richness. Shannon index and Simpson index to estimate  $\alpha$ -diversity of each soil sample. Coverage index was selected to calculate the percentage of these quenched total species in each soil sample.

**(iii).  $\beta$ -diversity analysis:** UniFrac principal coordinate analysis (PCoA) and unweighted pair group method with arithmetic mean (UPGMA) were calculated to estimate  $\beta$ -diversity of each soil sample.

**(iv). Correlation analysis between ginsenosides and soil microorganisms:** Redundancy analysis method (RDA) was constrained principal component analysis and classification method combining regression analysis and principal component analysis. It was used to show the co-relation between ginsenosides and microorganisms.

#### IV. Statistical analyses

SPSS version 16.0 was used for statistical analysis (SPSS Inc., Chicago, IL, USA). Means and SE were determined for all treatment replicates and subjected to ANOVA. Differentiate significant differences were tested by Duncan's tests and one-way analysis of variance among the four treatments. The significance level was chosen at  $P = 0.05$ .

## RESULTS AND DISCUSSION

### I. Change in microbial community

#### (i). Changes in Microbial community structure

**(a) Fungi :** At the phylum level, soil fungi detected were classified into six phyla, of which the total relative abundance of *Ascomycota*, *Basidiomycota*, *Unclassified* and *Mucoromycota* reached 99.9 %. The relative abundance of *Basidiomycetes* in CT was the highest, accounting for > 62.76 % of all the fungi in the soil (Fig. 1) and the relative abundance of *Ascomycetes* in ginseng rhizosphere soils was the highest, reaching 39-64 %. These results showed that the microbial community structure and composition of rhizosphere soil of ginseng significantly changed during its cultivation.

At the genus level, the soil fungi were classified into 403 taxa, of which 54 taxa had relative abundance > 1 % and 12 taxa with relative abundance > 3 %. The relative abundance of *Russula* was the highest (57.99 %) in CT and decreased significantly in GS2+2, GS2+3 and GS2+4 (Table 3). There are 8-genera with significantly increased relative abundance including *Leptodontidium*, *Fusarium*, *Neurospora*, *Cylindrocarpon*, *Coniochaeta*, *Cadophora*, *Trechispora* and *Solicoccozyma* in GS2+2, GS2+3 and GS 2+4. Of these, *Fusarium*, *Neurospora*, *Cylindrocarpon*, *Trechispora* and *Solicoccozyma*

were not detected in CT. Interestingly, the ginseng pathogenic fungi (*Fusarium* and *Cylindrocarpon*) population was significantly higher after planting ginseng but not detected in CT. This indicated that the rhizosphere soil microecological environment of ginseng was changed. The relative abundance of *Leptodontidium* was significantly higher in GS2+3 and GS2+4 compared with GS2+2 and CT. The *Fusarium*, *Coniochaeta* and *Trichoderma* relative abundances were significantly higher in GS2+2 than in GS2+3 and GS2+4 but were absent in CT. The results indicated that the fungal community structure and composition in the ginseng rhizosphere soils were correlated with cultivation ages.

**(b) Bacteria :** At the phylum level, the bacteria in soil samples were classified into 43 phyla by high-throughput sequencing, of which 12 were dominant phyla with abundance > 1 % (Fig. 1). Among these groups, the relative abundance of *Proteobacteria* was highest (44 %) in CT of all bacteria detected in the soil. The relative abundances of *Proteobacteria* and *Actinobacteria* decreased significantly in GS2+2, GS2+3 and GS 2+4 compared with CT. The relative abundance of *Actinomycetes* decreased from 18.07 % in CT to 9.20 %, 4.15 % and 14.08 % in GS2+2, GS2+3 and GS 2+4, respectively. In contrast, the phyla *Chloroflexi*, *Firmicutes* and *Verrucomicrobia* were enriched in GS2+2, GS2+3 and GS 2+4. The relative abundance of *Chloroflexi* increased by 210.73 %, 277.97 % and 99.62 %, respectively and the relative abundance of *Firmicutes* increased by 974.13 %, 732.76 % and 412.93 %, respectively in GS2+2, GS2+3 and GS 2+4 compared with CT.

The bacteria in each soil sample were classified into 623 taxa at the genus level, 65 of which had a relative abundance > 1 % and 29 of which had a relative abundance > 2 % (Tab. 2). There was little difference between the species of bacteria in the rhizosphere soil of ginseng and CT, but the bacterial community structure changed. There were 9 dominant genera (*Bradyrhizobium*, *Variibacter*, *Xanthobacteraceae\_ uncultured*, *Nitrosomonadaceae\_ uncultured*, *Haliangium*, *Subgroup 6\_norank*, *Bryobacter*, *Candidatus Solibacter*, *Acidimicrobiales\_norank*) with significantly reduced relative abundance. The genus *Acidobacteriaceae (Subgroup 1) uncultured*, *JG37-AG-4\_norank* and *HSB OF53-F07\_norank* were significantly higher after ginseng planting.

Li *et al.* (6) studied the soil microbial diversity of continuous cropping of ginseng using random amplified polymorphic DNA analysis. The results showed that the changes in soil ecological environment caused by ginseng growth were not conducive to actinomycetes, but were beneficial to fungi, which can reduce the antagonistic effects of soil microorganisms on the ginseng pathogens. The similar results were also reported by Zhang *et al.* (18). Xiao *et al.* (14) analyzed the microbial diversity of ginseng soil using PCR-DGGE and showed that the microbial community composition of ginseng soil changed significantly compared with forest soil. Among

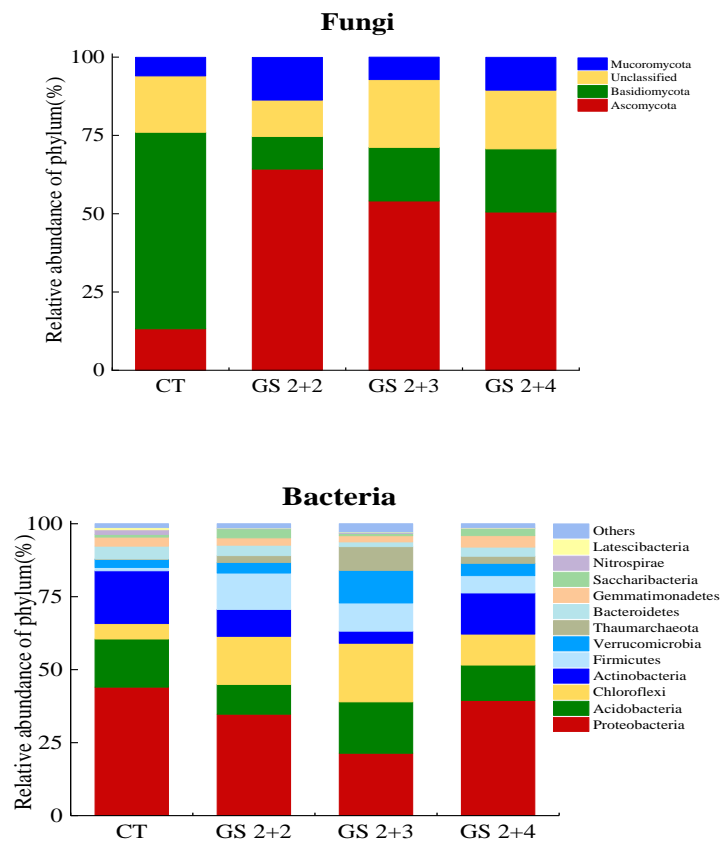


Figure 1. Bacterial and fungal community relative abundance in soil samples at phylum level. GS 2: Ginseng seeds were sown in forest soil and after 2 years, its seedlings were transplanted into new forest soils and cultivated for 2, 3, 4 years until harvest. This is the main cultivation pattern in ginseng-producing regions. CT: Forest soils, GS2+2: Ginseng soil after 2+2, GS2+3: Ginseng soil after 2+3 and GS2+4: Ginseng soil after 2+4 represent the ginseng rhizosphere soil after 2+2, 2+3 and 2+4 years, respectively.

them, the ginseng root rot fungus *Fusarium* was detected in all ginseng soil samples, while it was not detected in the forest soil control. Studies by the soil dilution plate method also found that with the extension of ginseng cultivation age, the number of bacteria and actinomycetes in the soils gradually decreased, while the number of fungi increased (2,5). The above reports are consistent with results presented in this article, as analyzed using the Illumina MiSeq platform. Most of the *Actinomycota* are beneficial to plant growth, which can antagonize ginseng pathogens. Therefore, the microbial community structure and composition changes in ginseng rhizosphere soils may be the main factor causing the serious ginseng root diseases during continuous cropping.

Table 2. The dominant genus of bacteria (relative abundance over 2 % at the genus level)

Phylum	Genus	CT	GS2+2	GS2+3	GS2+4
Proteobacteria	<i>Rhodanobacter</i>	1.15±0.38 <sup>b</sup>	12.87±6.49 <sup>a</sup>	1.74±0.42 <sup>b</sup>	8.19±0.7 <sup>ab</sup>
	<i>Rhizomicrobium</i>	1.6±0.16 <sup>b</sup>	1.39±0.06 <sup>b</sup>	1.66±0.05 <sup>b</sup>	5.17±0.86 <sup>a</sup>
	<i>Saccharibacteria_norank</i>	0.90±0.11 <sup>b</sup>	3.11±0.29 <sup>a</sup>	0.79±0.16 <sup>b</sup>	2.37±0.9 <sup>a</sup>
	<i>Bradyrhizobium</i>	4.16±0.6 <sup>a</sup>	0.58±0.07 <sup>b</sup>	0.91±0.04 <sup>b</sup>	1.15±0.15 <sup>b</sup>
	DA111_norank	1.37±0.36 <sup>a</sup>	1.23±0.36 <sup>a</sup>	1.72±0.11 <sup>a</sup>	2.37±0.87 <sup>a</sup>
	H16	1.78±0.37 <sup>a</sup>	0.88±0.70 <sup>a</sup>	1.44±0.34 <sup>a</sup>	2.34±1.62 <sup>a</sup>
	<i>Variibacter</i>	3.50±0.71 <sup>a</sup>	0.70±0.11 <sup>b</sup>	0.54±0.08 <sup>b</sup>	1.45±0.39 <sup>b</sup>
	<i>Xanthomonadales_norank</i>	1.40±0.17 <sup>b</sup>	0.25±0.02 <sup>c</sup>	1.03±0.16 <sup>b</sup>	3.16±0.89 <sup>a</sup>
	<i>Xanthobacteraceae_uncultured</i>	2.99±0.63 <sup>a</sup>	0.59±0.13 <sup>b</sup>	0.58±0.09 <sup>b</sup>	1.13±0.33 <sup>b</sup>
	<i>Nitrosomonadaceae_uncultured</i>	3.23±0.15 <sup>a</sup>	0.94±0.08 <sup>b</sup>	0.10±0.04 <sup>c</sup>	0.17±0.06 <sup>c</sup>
	<i>Haliangium</i>	2.30±0.59 <sup>a</sup>	0.45±0.02 <sup>b</sup>	0.30±0.06 <sup>b</sup>	0.60±0.12 <sup>b</sup>
Acidobacteria	<i>Acidobacteriaceae</i> (Subgroup 1)_uncultured	1.15±0.41 <sup>c</sup>	4.45±0.34 <sup>ab</sup>	5.41±0.93 <sup>a</sup>	3.16±0.57 <sup>b</sup>
	Subgroup 2_norank	0.95±0.24 <sup>b</sup>	1.99±0.34 <sup>b</sup>	5.48±1.42 <sup>a</sup>	3.2±1.62 <sup>ab</sup>
	Subgroup 6_norank	4.62±3.05 <sup>a</sup>	0.67±0.12 <sup>b</sup>	0.49±0.15 <sup>b</sup>	1.08±0.49 <sup>ab</sup>
	<i>Bryobacter</i>	2.5±0.66 <sup>a</sup>	0.89±0.11 <sup>b</sup>	1.52±0.34 <sup>ab</sup>	0.99±0.51 <sup>b</sup>
	<i>CandidatusSolibacter</i>	2.34±0.47 <sup>a</sup>	0.38±0.07 <sup>b</sup>	0.93±0.1 <sup>b</sup>	0.90±0.60 <sup>b</sup>
Chloroflexi	JG37-AG-4_norank	0.31±0.08 <sup>c</sup>	8.21±1.89 <sup>b</sup>	13.42±0.91 <sup>a</sup>	6.18±3.03 <sup>b</sup>
	HSB	0.15±0.09 <sup>d</sup>	4.15±0.41 <sup>a</sup>	1.70±0.32 <sup>b</sup>	1.05±0.09 <sup>c</sup>
	OF53-F07_norank	—	—	—	—
Actinobacteria	<i>Gaiellales_norank</i>	3.67±0.77 <sup>a</sup>	1.39±0.41 <sup>b</sup>	1.00±0.08 <sup>b</sup>	3.86±0.69 <sup>a</sup>
	<i>Acidimicrobiales_norank</i>	3.67±0.7 <sup>a</sup>	0.48±0.14 <sup>b</sup>	0.87±0.05 <sup>b</sup>	1.23±0.14 <sup>b</sup>
	<i>Acidothermus</i>	0.81±0.11 <sup>b</sup>	0.73±0.25 <sup>b</sup>	0.69±0.18 <sup>b</sup>	2.95±0.54 <sup>a</sup>
Firmicutes	<i>Bacillus</i>	0.53±0.08 <sup>b</sup>	2.49±0.37 <sup>ab</sup>	3.41±1.69 <sup>a</sup>	2.05±0.63 <sup>ab</sup>
	<i>Lactococcus</i>	0.37±0.02 <sup>b</sup>	1.17±0.1 <sup>ab</sup>	2.87±1.52 <sup>a</sup>	1.64±0.39 <sup>ab</sup>
	ODP1230B8.23_norank	0.07±0.04 <sup>b</sup>	1.2±0.53 <sup>ab</sup>	2.22±1.12 <sup>a</sup>	0.96±0.34 <sup>ab</sup>
	<i>Sporosarcina</i>	—	3.99±2.56 <sup>a</sup>	—	—
Verrucomicrobia	DA101 soil group_norank	1.78±1.2 <sup>b</sup>	2.99±0.54 <sup>b</sup>	7.88±1.7 <sup>a</sup>	2.96±1.59 <sup>b</sup>
	Thaumarchaeota	—	0.30±0.28 <sup>b</sup>	5.25±4.06 <sup>a</sup>	1.08±0.53 <sup>ab</sup>
	South African Gold Mine Gp 1(SAGMCG-1)_norank	—	—	—	—
	<i>CandidatusNitrosotalea</i>	0.01±0.01 <sup>a</sup>	1.63±1.34 <sup>a</sup>	2.27±1.72 <sup>a</sup>	1.00±0.58 <sup>a</sup>
Gemmatimonadetes	<i>Gemmatimonadaceae_uncultured</i>	2.35±0.42 <sup>ab</sup>	1.37±0.25 <sup>c</sup>	1.73±0.05 <sup>bc</sup>	3.24±0.61 <sup>a</sup>

GS 2: Ginseng seeds were sown in forest soil and after 2 years, its seedlings were transplanted into new forest soils and cultivated for 2, 3, 4 years until harvest. This is the main cultivation pattern in ginseng-producing regions. CT: Forest soils, GS2+2: Ginseng soil after 2+2, GS2+3: Ginseng soil after 2+3 and GS2+4: Ginseng soil after 2+4 represent the ginseng rhizosphere soil after 2+2, 2+3 and 2+4 years, respectively. Data are presented as means ± SEs (n = 3). Different lowercase letters indicate significant differences between soil samples (P<0.05).

Table 3. The dominant fungal genus (relative abundance over 3 % and significant changes at the genus level)

Phylum	Genus	CT	GS2+2	GS2+3	GS2+4
Ascomycota	<i>Leptodontidium</i>	0.40±0.07 <sup>b</sup>	3.69±0.85 <sup>ab</sup>	7.85±1.32 <sup>a</sup>	7.85±1.60 <sup>a</sup>
	<i>Fusarium</i>	—	14.75±10.93 <sup>a</sup>	0.04±11.08 <sup>b</sup>	0.15±0.12 <sup>b</sup>
	<i>Neurospora</i>	—	0.37±0.29 <sup>ab</sup>	0.33±0.32 <sup>ab</sup>	2.65±2.00 <sup>a</sup>
	<i>Ilyonectria</i>	0.01±0.00 <sup>a</sup>	1.74±1.30 <sup>a</sup>	1.33±1.34 <sup>a</sup>	1.50±2.08 <sup>a</sup>
	<i>Cylindrocarpon</i>	—	—	0.02±0.00 <sup>a</sup>	0.11±0.15 <sup>a</sup>
	<i>Coniochaeta</i>	0.02±0.00 <sup>b</sup>	4.60±2.80 <sup>a</sup>	0.04±1.65 <sup>b</sup>	1.66±1.86 <sup>ab</sup>
	<i>Cadophora</i>	0.04±0.02 <sup>b</sup>	0.07±0.06 <sup>b</sup>	3.45±0.03 <sup>a</sup>	0.57±0.03 <sup>b</sup>
	<i>Geomyces</i>	0.42±0.16 <sup>a</sup>	0.16±0.04 <sup>a</sup>	0.14±0.04 <sup>a</sup>	3.87±5.17 <sup>a</sup>
	<i>Trichoderma</i>	0.56±0.25 <sup>b</sup>	11.77±5.21 <sup>a</sup>	0.43±4.00 <sup>b</sup>	3.43±0.99 <sup>b</sup>
	<i>Cladophialophora</i>	0.75±0.26 <sup>a</sup>	0.87±0.28 <sup>a</sup>	11.47±0.20 <sup>a</sup>	2.96±0.25 <sup>a</sup>
	<i>Penicillium</i>	5.02±6.80 <sup>a</sup>	6.56±2.49 <sup>a</sup>	0.60±4.64 <sup>a</sup>	2.96±2.86 <sup>a</sup>
Basidiomycota	<i>Russula</i>	57.99±6.19 <sup>a</sup>	0.61±0.41 <sup>b</sup>	0.13±24.83 <sup>b</sup>	1.14±1.15 <sup>b</sup>
	<i>Trechispora</i>	—	—	30.4±0.00 <sup>a</sup>	4.04±2.09 <sup>a</sup>
	<i>Ceratobasidium</i>	0.47±0.19 <sup>a</sup>	0.74±0.14 <sup>a</sup>	3.84±0.29 <sup>a</sup>	4.66±3.28 <sup>a</sup>
	<i>Solicoccozyma</i>	—	0.06±0.04 <sup>a</sup>	0.49±0.04 <sup>a</sup>	2.14±2.96 <sup>a</sup>
Mucoromycota	<i>Mortierella</i>	5.93±1.61 <sup>a</sup>	11.37±6.51 <sup>a</sup>	4.68±4.51 <sup>a</sup>	8.48±7.12 <sup>a</sup>

GS 2: Ginseng seeds were sown in forest soil and after 2 years, its seedlings were transplanted into new forest soils and cultivated for 2, 3, 4 years until harvest. This is the main cultivation pattern in ginseng-producing regions. CT: Forest soils, GS2+2: Ginseng soil after 2+2, GS2+3: Ginseng soil after 2+3 and GS2+4: Ginseng soil after 2+4 represent the ginseng rhizosphere soil after 2+2, 2+3 and 2+4 years, respectively. Data are presented as means ± SEs (n = 3). Different lowercase letters indicate significant differences between soil samples (P<0.05).

### (ii). Microbial community $\alpha$ -diversity changes

Five indexes of soil microorganisms were analyzed (Table 4). According to 97 % sequence similarity, the number of Operational Taxonomic Unit (OTU) and community richness index Chao1 in ginseng rhizosphere soil (GS2+2, GS2+3, GS2+4) was less than in CT, but the differences were not significant. The Simpson index of ginseng soil bacteria was slightly higher than CT and Shannon index was slightly lower than CT, indicating that the bacterial community diversity of ginseng rhizosphere soil has declined slightly, but the differences between the samples were not significant. Coverage index of bacteria was >0.97, which reflected the high reliability of the sequencing results.

According to 97 % sequence similarity, the number of OTU of fungi in ginseng rhizosphere soil (GS2+2, GS2+3, GS2+4) was significantly higher than in CT. Chao1

Table 4. Microbial diversity indices of soil samples

Microbe	Samples	OTU quantity	Chao1 index	Shannon index	Simpson index	Coverage
Bacteria	CT	2352.7±120.52 <sup>a</sup>	2713.7±90.98 <sup>ab</sup>	6.67±0.08 <sup>a</sup>	0.003±0.000 <sup>b</sup>	0.986±0.004 <sup>a</sup>
	GS2+2	2044.0±85.11 <sup>a</sup>	2527.3±110.39 <sup>b</sup>	6.15±0.17 <sup>a</sup>	0.007±0.002 <sup>ab</sup>	0.988±0.004 <sup>a</sup>
	GS2+3	2269.0±306.77 <sup>a</sup>	2628.0±29.87 <sup>ab</sup>	6.12±0.51 <sup>a</sup>	0.010±0.006 <sup>a</sup>	0.991±0.008 <sup>a</sup>
	GS2+4	2287.7±47.06 <sup>a</sup>	2776.3±148.45 <sup>a</sup>	6.28±0.23 <sup>a</sup>	0.006±0.001 <sup>ab</sup>	0.987±0.003 <sup>a</sup>
Fungi	CT	272.7±24.12 <sup>b</sup>	310.0±39.30 <sup>b</sup>	2.07±0.12 <sup>b</sup>	0.35±0.06 <sup>a</sup>	0.999±0.000 <sup>a</sup>
	GS2+2	453.7±145.52 <sup>ab</sup>	482.7±124.0 <sup>ab</sup>	3.54±1.48 <sup>a</sup>	0.13±0.16 <sup>b</sup>	0.999±0.000 <sup>a</sup>
	GS2+3	590.7±186.62 <sup>a</sup>	799.7±295.1 <sup>a</sup>	4.43±0.36 <sup>a</sup>	0.04±0.03 <sup>b</sup>	0.996±0.002 <sup>b</sup>
	GS2+4	598.3±140.97 <sup>a</sup>	652.3±124.8 <sup>a</sup>	4.59±0.19 <sup>a</sup>	0.03±0.01 <sup>b</sup>	0.999±0.001 <sup>a</sup>

GS 2: Ginseng seeds were sown in forest soil and after 2 years, its seedlings were transplanted into new forest soils and cultivated for 2, 3, 4 years until harvest. This is the main cultivation pattern in ginseng-producing regions. CT: Forest soils, GS2+2: Ginseng soil after 2+2, GS2+3: Ginseng soil after 2+3 and GS2+4: Ginseng soil after 2+4 represent the ginseng rhizosphere soil after 2+2, 2+3 and 2+4 years, respectively. Data are presented as means ± SEs (n = 3). Different lowercase letters indicate significant differences between soil samples (P < 0.05).

index showed that the abundance of fungal community in rhizosphere soil of ginseng was significantly higher than in CT. Shannon index and Simpson index showed that fungal community diversity in ginseng rhizosphere soil was significantly higher than in CT, but the differences among GS2+2, GS2+3 and GS 2+4 were not significant. Coverage index of fungi was >0.99, indicating that the sequencing results were trusted and reflected the real situation of fungus in the sample. Xiao *et al.* (14) have also reported that the ginseng cultivation decreased the bacterial diversity and increased the fungal diversity in soils with planting age, which is consistent with the results of this study.

### (iii). Microbial community $\beta$ -diversity changes

The similarities and differences in bacterial and fungal evolution in various soil samples were compared by unifracs PCoA and UPGMA cluster analysis (Fig. 2, 3, 4). Based on unifracs PCoA analysis, the bacterial and fungal diversity of soil samples was divided into two groups. GS2+2, GS2+3 and GS 2+4 were divided into one group and CT was divided into another group. GS2+3 and GS 2+4 were closer together, while GS2+2, GS2+3 and GS 2+4 differed well. The contribution rates of principal components 1 (PC1), 2 (PC2) and 3 (PC3) in bacteria were 46.84 %, 17.71 % and 12.26 %, respectively, and those in fungi were 31.59 %, 26.38 % and 12.01 %, respectively, indicating that ginseng cultivation was the main influencing factor and cultivation age was the secondary influencing factor.

Hierarchical clustering analysis revealed that the microbial communities in GS2+3 and GS 2+4 were similar. The community structures resulting from GS2+2, GS2+3 and GS 2+4 were clustered together, but these groups were separated from CT group. In addition, the group GS 2+4 group displayed the largest differences from CT. These results indicated that the structures and compositions of bacterial and fungal community in soil samples were significantly affected by the ginseng growth process.

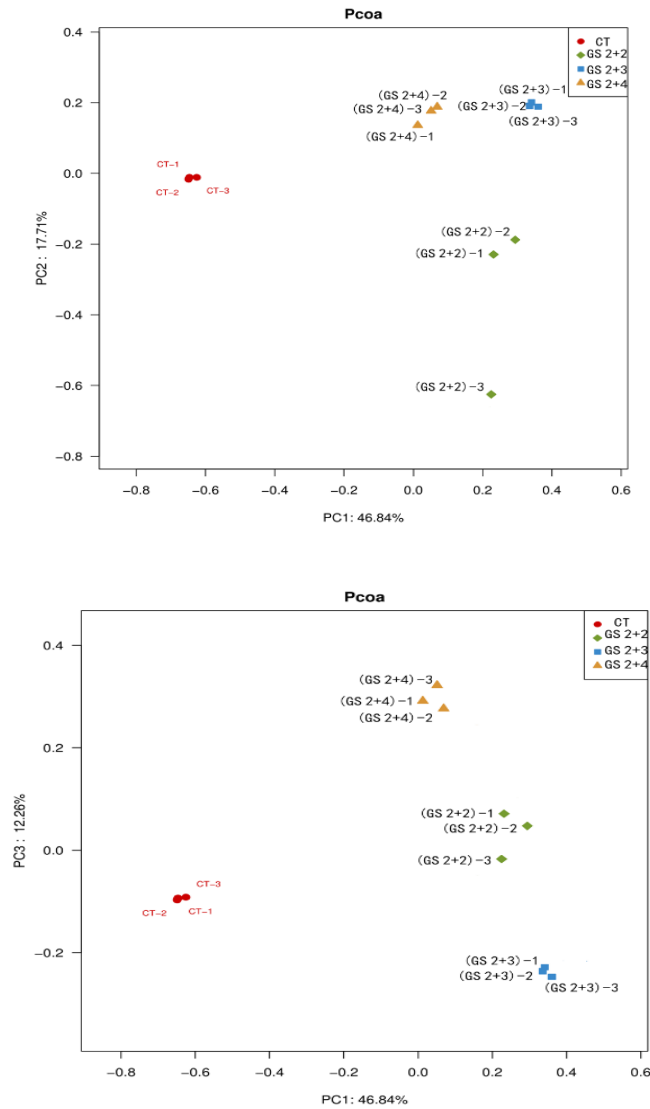


Figure 2. PCoA diagram of bacteria from soil samples. GS 2: Ginseng seeds were sown in forest soil and after 2 years, its seedlings were transplanted into new forest soils and cultivated for 2, 3, 4 years until harvest. This is the main cultivation pattern in ginseng-producing regions. CT: Forest soils, GS2+2: Ginseng soil after 2+2, GS2+3: Ginseng soil after 2+3 and GS2+4: Ginseng soil after 2+4 represent the ginseng rhizosphere soil after 2+2, 2+3 and 2+4 years, respectively.

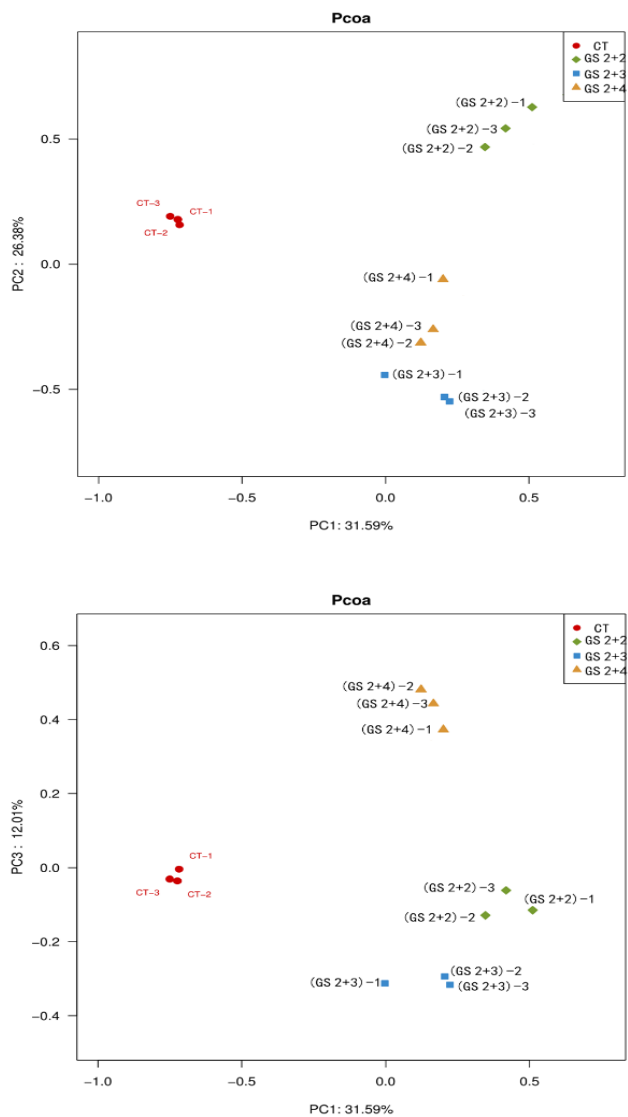


Figure 3. PCoA diagram of fungi from soil samples. CT: Forest soils, GS 2: Ginseng seeds were sown in forest soil and after 2 years, its seedlings were transplanted into new forest soils and cultivated for 2, 3, 4 years until harvest. This is the main cultivation pattern in ginseng-producing regions. CT: Forest soils, GS2+2: Ginseng soil after 2+2, GS2+3: Ginseng soil after 2+3 and GS2+4: Ginseng soil after 2+4 represent the ginseng rhizosphere soil after 2+2, 2+3 and 2+4 years, respectively.

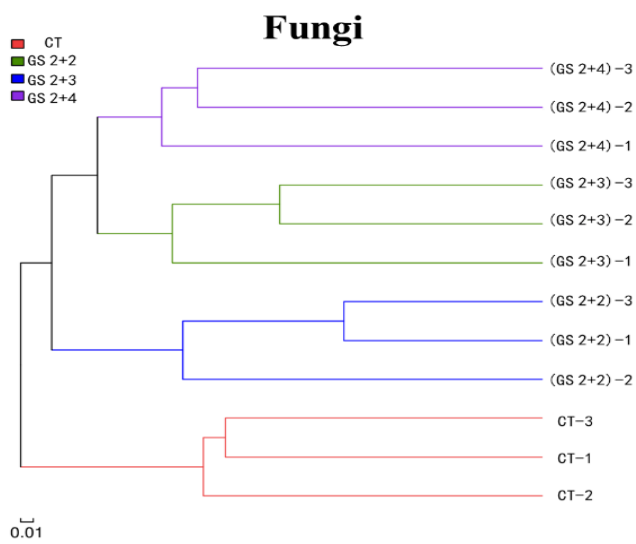
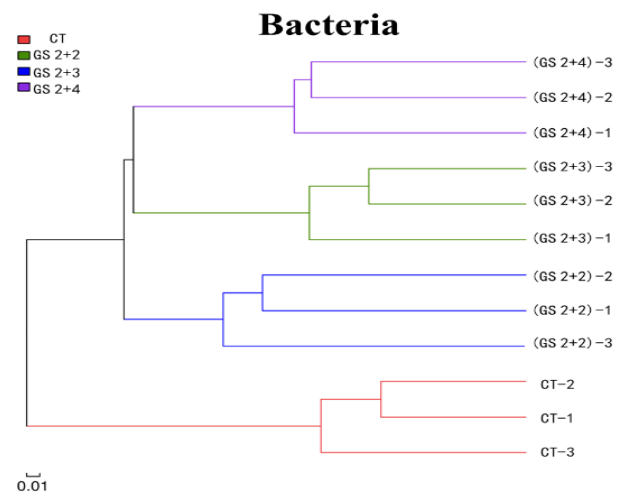


Figure 4. UPGMA Cluster tree of bacteria and fungi from soil samples

## II. Ginsenosides contents in soil

Ginsenosides are a group of dammarane-type triterpenoid saponins produced by *Panax* plants. To date, more than 37 ginsenosides have been isolated from *P. ginseng* roots which accounts for 3-7 % of root dry weight (11). Based on their saponins, the major ginsenosides are classified into the following two groups: (i). Protopanaxadiol (PPD)-type saponins (e.g., ginsenosides Rb<sub>1</sub>, Rb<sub>2</sub>, Rc and Rd) and (ii). Protopanaxatriol (PPT)-type saponins (e.g., ginsenosides Rg<sub>1</sub> and Re). The ginsenosides Rb<sub>1</sub>, Rb<sub>2</sub>, Rc, Rd, Rg<sub>1</sub> and Re, the main components of ginseng root saponins are > 90 % of the total content of saponins (8). Previous studies have found that ginsenoside Rb<sub>1</sub> and Rb<sub>2</sub> promote the growth of ginseng root pathogens, while ginseng pathogens and some non-pathogens can degrade the ginsenosides Rb<sub>1</sub>, Rb<sub>2</sub>, Rc and Rd through extracellular enzymes. The degradation path of ginsenoside Rb<sub>1</sub>, which has the highest content in *P. ginseng* root is Rb<sub>1</sub>→G-VXII/Rd→F<sub>2</sub> (9). Accordingly, the contents of ginsenoside monomers Rb<sub>1</sub>, Rb<sub>2</sub>, Rc, Rd, Rg<sub>1</sub>, Re, G-VXII and F<sub>2</sub> in soil samples were determined first using UPLC-MS/MS. The contents of 8 kinds of ginsenoside monomers were very low or not detected in CT, while those in GS2+2, GS2+3 and GS2+4 were detected significantly, among which the Rd content was highest (Table 5). The relative contents of ginsenosides Rb<sub>1</sub>, Rb<sub>2</sub> and Rc, Rg<sub>1</sub> and Re decreased, while the relative contents of ginsenosides G-VXII and F<sub>2</sub> increased in GS2+3 and GS2+4 compared with GS2+2. The above results indicated that ginseng roots secreted the ginsenosides into the soil during the growth and the relative contents of ginsenosides in soils secreted by ginseng roots decreased with the increase of cultivation age. This change of ginsenosides relative contents as ginseng ages was positively correlated with the relative abundances of *Fusarium*, *Coniochaeta* and *Trichoderma* in ginseng rhizosphere soils. On the other hand, the relative contents of ginsenosides G-VXII and F<sub>2</sub>, which were the

Table 5. The content and ratio of ginsenosides in soils

Data types	representations	CT	GS2+2	GS2+3	GS2+4
Ginsenoside content (ng·g <sup>-1</sup> )	Rb <sub>1</sub>	0.04±0.03	20.47±2.44	1.25±0.36	1.97±1.06
	Rb <sub>2</sub>	0.04±0.03	15.75±1.98	0.44±0.14	1.09±0.34
	Rc	0.04±0.03	17.03±3.08	0.48±0.17	1.23±0.45
	Rd	0.05±0.05	22.41±4.08	4.69±2.16	5.13±2.38
	Re	0.01±0.02	14.81±2.13	0.29±0.05	0.83±0.43
	Rg <sub>1</sub>	—	2.44±0.34	0.08±0.03	0.29±0.12
	G-VXII	—	2.83±0.36	0.32±0.09	1.11±0.66
	F <sub>2</sub>	—	2.15±0.94	1.11±0.36	0.71±0.40
	TG	0.19±0.16	97.88±12.87	8.67±3.24	12.36±5.73
Ratio (%)	(Rb <sub>1</sub> +Rb <sub>2</sub> +Rc)/TG	45.00	54.38	26.80	35.12
	(Re+Rg <sub>1</sub> )/TG	3.33	17.69	4.75	8.97
	G-VXII/TG	—	2.93	3.93	8.59
	F <sub>2</sub> /TG	—	2.17	13.14	5.57

TG : Total content of 8 kinds of ginsenosides. GS 2: Ginseng seeds were sown in forest soil and after 2 years, its seedlings were transplanted into new forest soils and cultivated for 2, 3, 4 years until harvest. This is the main cultivation pattern in ginseng-producing regions. CT: Forest soils, GS2+2: Ginseng soil after 2+2, GS2+3: Ginseng soil after 2+3 and GS2+4 : Ginseng soil after 2+4 represent the ginseng rhizosphere soil after 2+2, 2+3 and 2+4 years, respectively.

degradation products of ginsenoside Rb<sub>1</sub> by soil fungi increased with the increase of cultivation ages. The above high-throughput sequencing results showed that the relative abundances of fungal communities, especially these ginseng pathogenic fungi (*Fusarium* and *Cylindrocarpon*) increased significantly in the ginseng rhizosphere soils. These may increase the degradation of ginsenosides Rb<sub>1</sub>, Rb<sub>2</sub> and Rc to produce ginsenosides Rd, G-VXII and F<sub>2</sub> in the rhizosphere soil of ginseng during its growth. Changes in the microbial communities may result in variations in soil metabolic capacity, biodegradation and disease suppression (1).

### III. RDA of soil microorganisms and ginsenosides:

The relationship between 8-ginsenosides (Rb<sub>1</sub>, Rb<sub>2</sub>, Rc, Rd, Rg<sub>1</sub>, Re, G-VXII and F<sub>2</sub>) and 10-soil microorganisms (Bacteria: JG37-AG-4\_norank, *Rhodanobacter*, DA101 soil group\_norank, Acidobacteriaceae (Subgroup 1)\_uncultured, Subgroup 2\_norank, Gaiellales\_norank, *Rhizomicrobium*, Gemmatimonadaceae\_uncultured, *Bacillus* and *Saccharibacteria\_norank*; Fungi: *Russula*, *Trechispora*, *Mortierella*, *Leptodontidium*, *Trichoderma*, *Cladophialophora*, *Penicillium*, *Fusarium*, *Ceratobasidium* and *Coniochaeta*) with the highest relative abundances were obtained by RDA analysis (Figs. 5,6). The Arrows represent environmental factors. The quadrant of the arrow indicates the positive and negative correlation between environmental factors and the sorting axis. The length of the connecting line between the arrow and the origin represents the degree of correlation between an environmental factor and community distribution and species distribution. The longer line means the greater correlation. The angle between the arrow line and the sorting axis indicates the correlation between an environmental factor and the sorting axis. The smaller angle means less correlation. The acute angle represents a positive correlation, the right angle shows irrelevant and the obtuse angle indicates a negative correlation. According to RDA sequencing diagram, the first two sequencing axes accounted for 78.03 % of bacteria and ginsenosides (Fig. 5). Ginsenosides were distributed in the first quadrant, which was positively correlated with PC1 and PC2. The correlation between ginsenosides and sequencing axis followed the order: F<sub>2</sub> > R<sub>d</sub> > Rb<sub>1</sub> > Rb<sub>2</sub> > Rc > G-VXII > Re > Rg<sub>1</sub>. *Bacillus*, *JG37-Ag-4\_Norank*, *Acidobacteriaceae (Subgroup 1)-Uncultured* were positively correlated with ginsenosides. *Gaiellales\_norank* is negatively correlated with F<sub>2</sub>.

The first two sequencing axes accounted for 76.63 % of fungi and ginsenosides (Fig. 6). Ginsenosides are distributed in the first quadrant in the RDA diagram, which was positively correlated with PC1 and PC2. The correlation between ginsenosides and sequencing axis followed the order: Rg<sub>1</sub> > Re > Rb<sub>2</sub> > Rc > G-VXII > Rb<sub>1</sub> > F<sub>2</sub> > R<sub>d</sub>. The *Coniochaeta*, *Trechispora*, *Fusarium*, *Mortierella* and ginsenosides showed positive correlation. *Trechispora*, *Cladophialophora*, *Ceratobasidium* and *Leptodontidium* had variable degrees of positive correlation with F<sub>2</sub> and Rd, but have different degrees of negative correlation with Rg<sub>1</sub>, Re, Rb<sub>2</sub>, Rc and G-VXII. *Russula* was negatively correlated with ginsenosides.

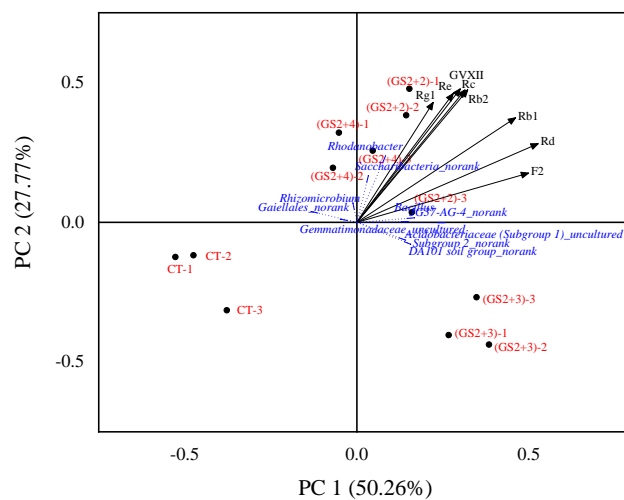


Figure 5. RDA diagram of bacteria and ginsenosides (The solid line represents ginsenosides and the dashed line represents species)

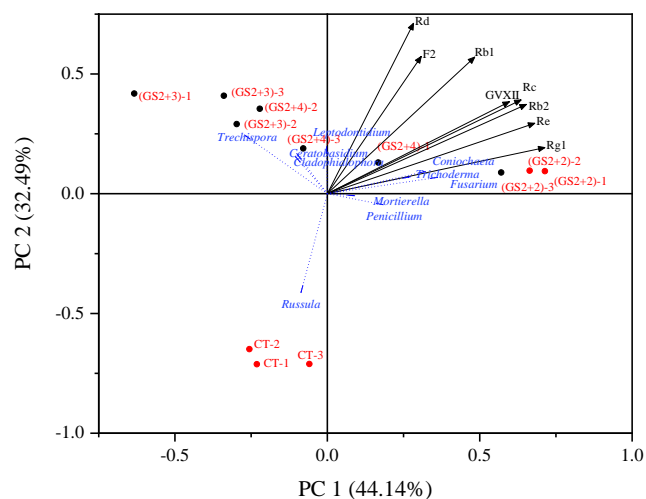


Figure 6. RDA diagram of fungi and ginsenosides (The solid line represents ginsenosides and the dashed line represents species)

## CONCLUSIONS

Significant changes in composition and structure of fungal community in ginseng rhizosphere soil were observed. There was significant increase in diversity of fungal community in the rhizosphere soil of ginseng compared to control forest soil. The relative abundance of *Ascomycota* increased significantly, while, the relative abundance of *Basidiomycetes* decreased in ginseng rhizosphere soil. The ginseng pathogenic fungi (*Fusarium* and *Cylindrocarpon*) were detected in the ginseng rhizosphere soils than in forest soil. The bacterial community diversity in ginseng rhizosphere soil was slightly lower than forest soil, but the differences were not significant. The bacterial community structure was significantly changed after the ginseng cultivation. The relative abundances of *Chloroflexi*, *Firmicutes* and *Verrucomicrobia* increased significantly, while these *Proteobacteria* and *Actinobacteria* decreased significantly in ginseng rhizosphere soils compared with CT. The relative contents of ginsenosides (Rb<sub>1</sub>, Rb<sub>2</sub> and Rc) secreted by ginseng roots decreased, while, these ginsenosides degradation products (Rd, G-VXII and F<sub>2</sub>) increased in ginseng rhizosphere soils during the ginseng growth. The allelopathy of *P. ginseng* on soil microorganisms mediated via ginsenosides secreted by its roots was a key factor resulting in microecological imbalance in ginseng rhizosphere soil.

## ACKNOWLEDGMENTS

This study was financially supported by the National Natural Science Foundation of China (No.31870339).

## CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

## REFERENCES

1. Dong, L.L., Xu, J., Li, Y., Fang, H.L., Niu, W.H., Li, X.W., Zhang, Y.J., Ding, W.L. and Chen, S.L. (2018). Manipulation of microbial community in the rhizosphere alleviates the replanting issues in *Panax ginseng*. *Soil Biology and Biochemistry* **125**: 64-74.
2. Guo, S.H., Wang, Y.X., Geng, Y.Q. and Wang, Y.Z. (1990). Effects on the ecological distribution of soil microorganisms by ginseng-cultivating. *Acta Ecologica Sinica* **10**: 286-287. (Chinese).
3. Heuberger, H., Bauer, R., Friedl, F., Heubl, G., Hummelsberger, J., Nögel, R., Seidenberger, R. and Torres-Londoño, P. (2010). Cultivation and breeding of Chinese medicinal plants in Germany. *Planta Medica* **76**: 1956-1962.
4. Ivanov, D.A., Georgakopoulos, Jorge, R.C. and Bernards, M.A. (2016). The chemo-attractant potential of ginsenosides in the Ginseng-*Pythium irregulare* pathosystem. *Phytochemistry* **122**: 56-64.
5. Li, Y., Liu, S.L. and Ding, W.L. (2009). Allelopathy of ginseng root exudates on pathogens of ginseng. *Acta Ecologica Sinica* **29**: 161-168. (Chinese).
6. Li, Y., Ying, Y.X., Zhao, D.Y. and Ding, W.L. (2012). Microbial community diversity analysis of *Panax ginseng* rhizosphere and non-rhizosphere soil using randomly amplified polymorphic DNA method. *Open Journal of Genetics* **2**: 95-102.

7. Lu, R.K. (2000). *The Analysis Method of Soil Agricultural Chemistry*. China Agriculture Sciencetech Press, Beijing, pp. 109-194. (Chinese).
8. Ma, X.Q., Liang, X.M., Xu, Q., Zhang, X.Z and Xiao, H.B. (2010). Identification of ginsenosides in roots of *Panax ginseng* by HPLC-APCI/MS. *Phytochemical Analysis* **16**: 181-187.
9. Nicol, R.W., Traquair, J.A. and Bernards, M.A. (2002). Ginsenosides as host resistance factors in American ginseng (*Panax quinquefolius*). *Canadian Journal of Botany* **80**: 557-562.
10. Nicol, R.W., Yousef, L.F., Traquair, J.A. and Bernards, M.A., (2003). Ginsenosides stimulate the growth of soil-borne pathogens of American ginseng. *Phytochemistry* **64**: 257-264.
11. Qi, L.W., Wang, C.Z. and Yuan, C.S. (2011). Isolation and analysis of ginseng: Advances and challenges. *Natural Product Reports* **28**: 467-495.
12. Shi, K., Lei, F.J., Xu, Y.H., Liu, F.F., Yang, H., Zhang, A.H. and Zhang, L.X. (2016). Chemotaxis response of *Fusarium solani* and *Cylindrocarpon destructans* on total ginsenosides. *Chinese Traditional and Herbal Drugs* **47**: 821-826. (Chinese).
13. Wang, J., Chen, H.L., Gao, J., Guo, J.X., Zhao, X.S. and Zhou, Y.F. (2018). Ginsenosides and ginsenosidases in the pathobiology of ginseng-*Cylindrocarpon destructans* (Zinss) Scholten. *Plant Physiology and Biochemistry* **123**: 406-413.
14. Xiao, C.P., Yang, L.M., Zhang, L.X., Liu, C.J. and Han, M. (2016). Effects of cultivation ages and modes on microbial diversity in the rhizosphere soil of *Panax ginseng*. *Journal of Ginseng Research* **40**: 28-37.
15. Yousef, L.F. and Bernards, M.A. (2006). *In-vitro* metabolism of ginsenosides by the ginseng root pathogen *Pythium irregulare*. *Phytochemistry* **67**: 1740-1749.
16. Zhang, A.H., Shi, K., Xu, Y.H., Sun, J.M. and Lei, F.J. (2016). Chemotaxis response of *Rhizoctonia solani* and *Sclerotinia schinsengto* total ginsenosides. *Journal of Northwest A&F University* (Natural Science Edition) **44**: 200-204. (Chinese).
17. Zhang, B., Peng, Y., Zhang, Z., Liu, H., Qi, Y., Liu, S. and Xiao, P. (2010). GAP production of TCM herbs in China, *Planta Medica* **76**: 1948-1955.
18. Zhang, H.Y. (2014). *Study on Microecology Mechanism of Ginseng Replant Root Disease and Actinomycetes Restoration*. Ph.D. Dissertation. pp. 42. Northwest A&F University, Shaanxi (Chinese).
19. Zhao, X.S., Gao, J., Song, C.C., Fang, Q., Wang, N., Zhao, T.J., Liu, D.B. and Zhou, Y.F. (2012). Fungal sensitivity to and enzymatic deglycosylation of the ginsenosides. *Phytochemistry* **78**: 65-71.

## PUBLISHER NOTE

Allelopathy Journal remains neutral with regard to jurisdictional claims in published Maps and Institutional Affiliations.