



Original article

Effects of phenolic acids from ginseng rhizosphere on soil fungi structure, richness and diversity in consecutive monoculturing of ginseng



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ABSTRACT

Ginseng yield and quality are seriously compromised by consecutive monoculturing in northeastern China. The imbalance of soil fungi communities and autotoxicity of ginseng are the major factors in consecutive monoculturing ginseng crops. Soil fungal communities were identified using Illumina MiSeq sequencing, applied to soils that consecutively cultured ginseng (CCG) for six years and new forest soil (NFS), or receiving application of phenolic acids (PAs). The CCG field received five treatments with five different phenolic acids, including gallic acid (GA), salicylic acid (SA), 3-phenylpropionic acid (3-PA), benzoic acid (BA) and cinnamic acid (CA), which were detected from ginseng rhizosphere in consecutive cropping soil. Fungal richness, fungi diversity, community composition, relative taxon abundances, root rot disease, and growth rate were compared among the different treatments. 579 fungal operational taxonomic units at 97% ITS sequence identity were found among 201,617 sequence reads derived from 18 separate soil samples. Members of the phylum *Ascomycota* dominated the soil fungal communities, and putative pathogens, such as *Fusarium*, *Gibberella* and *Nectriaceae_unclassified* which may include the abundant sexual morph of *Cylindrocarpon destructans*, showed higher relative abundances in the CCG fields. Compared to the CCG and NFS fields, PAs (except CA) enhanced the fungi richness and decreased fungi diversity. Cluster analysis indicated that the PAs (except CA) changed the fungi structure in a uniform way. PAs stimulate root rot disease and enhance disease severity, restricting plant growth. The results suggest that the PAs (except CA) may enhance the fungi richness, decrease the fungi diversity and changed the fungi structure to increase fungal pathogen loads, which could explain the declined yield and quality of ginseng in consecutively monocultured ginseng crops.

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1. Introduction

Panax ginseng Meyer (Araliaceae) was one of the most well known Chinese herbal medicines and wild plants grown in the northeastern region of China (You et al., 2015). In recent years, ginseng was mainly dependent on artificial cultivation in China. But this method of cultivation was severely aggravated by continuous cropping obstacles, including *Cylindrocarpon* root rot caused by *Cylindrocarpon destructans* (Zinns) Scholten, which is the most

severe soil-borne disease and is difficult to control in ginseng fields (Akin et al., 2017; Banerjee et al., 2017; Demir et al., 2017; Khan et al., 2018). This disease becomes more serious after continuous cropping and the symptoms can be observed at all developmental stages of the plant (Rahman and Punja-Z, 2005; Yan and Fu, 2002) and results in significant yield decline at last (Dou et al., 1996). In brief, ginseng cannot be cultivated on the same plot of land for several consecutive years. The roots turn rot on account of soil borne disease (Han et al., 1998). In the development of ginseng industries, large-scale deforestation increasingly occurred, not only damaging forest resources but also limiting sustainable development of the environment (Zhao et al., 2012). Hence, the contradiction between ginseng industries and environmental conservation has become a major problem that needs to be solved urgently (Alhawassi et al., 2018; Gao et al., 2017; Khan et al., 2018a, 2018b; Yang et al., 2017). Previous studies indicate that four major factors, including imbalance of soil microbial community (Xie and

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Xu, 1996), autotoxicity of ginseng (Zhao, 1995), deterioration of soil physicochemical characteristics (Zhao et al., 1999), and soil borne diseases (He et al., 2009), contribute to discontinuous cultivation of ginseng. In the past several years, some researchers have investigated soil improvement, sterilization and autotoxicity, but the underlying mechanisms between soil microbial diversity and ginseng cultivation are still poorly understood (Xiao et al., 2014).

Soil microorganisms play a vital role for soil functions, such as mineral nutrient cycling, organic matter turnover, and soil structure formation (Brussaard et al., 2007). Fungi are an important group of soil microorganisms (Fierer et al., 2007) and comprise multiple functional groups, such as decomposers and mycorrhizal fungi (Stajich et al., 2009; Hibbett et al., 2001; Yu et al., 2012). While many factors could contribute to increased disease pressure and yield decline in consecutive ginseng monoculturing, shifts in soil fungal communities are a very likely factor (Li et al., 2012).

Allelochemicals released by plant leaching, root exudation, volatilization, and residue decomposition have mostly negative effects on crops (Weir et al., 2004; Jilani et al., 2008). The allelochemicals produced by ginseng are closely related to continuous monoculture cropping systems, including esters, quinones, phenolic acids (PAs), and alkanes (Zhao et al., 1999; Li et al., 2009; Liu et al., 2015). Previous experiments have shown that soil PAs can inhibit or delay germination and growth in crops of the same species (Ben-Hammouda et al., 2002; Ill-Min and David, 2000), which is important for crops such as carrot, muskmelon, and American ginseng (Jasicka-Misiak et al., 2005; Jiao et al., 2015; Yang et al., 2014). PAs are directly exuded into the rhizosphere environment, which initiates and manipulates biological interactions between roots and soil microorganisms and, thus, plays an active role in root–microbe communication (Yu and Matsui 1994; Yu et al., 2003).

Previous studies of the fungi communities were based on conventional isolation culturing and 16S/18S rRNA gene fingerprinting, such as denaturing gradient gel electrophoresis (DGGE), which only detects certain dominant microbial groups (Li et al., 2012; Yasufumi et al., 2012; Zhou and Wu, 2012). High-throughput microbial community sequencing on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) is an effective method for studying environmental microbial communities (Caporaso et al., 2012). This platform is not only able to analyze V2 or V4 variable regions from microbial 16S rRNA (Baker et al., 2003; Broadhurst et al., 2012; Roden et al., 2012), but the technique also allows for enhanced sequencing speed and sequencing throughput (Hu et al., 2004). Most studies on fungi have only targeted the ITS1 region as a DNA metabarcode (Nilsson et al., 2009; Lumini et al., 2010; Xu et al., 2012; Yu et al., 2013). The release of the Illumina MiSeq platform, which extends the length of the sequence reads to 600 bp, has recently enabled analyses of the potentially more informative ITS2 region (Amend et al. 2010). Therefore, ITS1 and ITS2 recently became targets of DNA metabarcoding of soil fungal communities (Schoch et al. 2012; Toju et al. 2012).

We identified 5 PAs from ginseng rhizosphere soil: gallic acid (GA), salicylic acid (SA), 3-phenylpropionic acid (3-PA), benzoic acid (BA), and cinnamic acid (CA) (Li et al., 2016). We found GA, SA, and BA at a concentration of 0.5 mmol·L⁻¹, and the concentrations of 3-PA and CA were 0.05 mmol·L⁻¹. These concentrations significantly enhanced the spore germination ($P < 0.05$), mycelium growth in petri dishes, and aggravated the disease severity of *Cylindrocarpum* root rot of ginseng.

This study aims to provide the baseline data about the phenolic acids from ginseng rhizosphere altered the soil fungi structure, richness and diversity in consecutive monoculturing of ginseng.

2. Material and methods

2.1. Site description and soil sampling

The study was conducted in field beds (each 20 m in length, 1.5 m in width and 30 cm in height) in Shenyang Agricultural University, Shenyang, Liaoning province (28°130'N, 116°55'E) during 3 successive seasons (2012–2015). Soil for the new forest soil (NFS) treatment used for field beds was collected from the new forest field in Taiwang country, Ji'an, Jilin province, China. The other treatment soils were collected from the beds of ginseng plots continuously monocultivated for 6 years near by the new forest field at the same place. Ginseng was planted on this field for the first time in April 2005 and harvested in October 2011. The characteristics of the NFS treatment soils used for field beds were as follows: pH, 5.7; 30.6 g·kg⁻¹ organic matter content; 130.7 mg·kg⁻¹ available N; 46.8 mg·kg⁻¹ available P; 399.7 mg·kg⁻¹ available K. The 2-year-old healthy ginseng seedlings used for field experiments were purchased from Xinbin county, Fushun, Liaoning province, China. Plastic covers and shading nets were used over the beds from May through to September. Straw curtains covered the beds to maintain stable soil moisture and temperature.

Seven treatments (NFS, CCG, GA, SA, BA, 3-PA, and CA) were selected for this study. The NFS treatment involved ginseng planted with new forest soil and non-amended PAs but equal volume sterile water (CK). The CCG treatment involved ginseng planted with continuously monocultivated (for 6 years) and non-amended PAs but equal volume sterile water. The other treatments involved ginseng planted with the new forest soil and amended solution GA at 0.053 mmol·L⁻¹, SA at 0.519 mmol·L⁻¹, BA at 0.137 mmol·L⁻¹, 3-PA at 0.175 mmol·L⁻¹ and CA at 0.061 mmol·L⁻¹ with 100 mL every 7 days to keep the contents stable during three successive seasons respectively (Li et al., 2016).

Soil samples were collected in October 2015. Rhizosphere soils attached to the root of ginseng to about 2 mm thickness were collected from five random ginseng plants with three replications in each treatment. The five soil samples of each replication were mixed uniformly to obtain a single homogeneous sample thoroughly for soil analysis. Upon arrival to the lab the soil samples were immediately frozen at –80 °C.

2.2. DNA extraction and PCR amplification

The total soil DNA from 0.25 g soil subsamples were extracted according to manufacturer's protocols, using the soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.). We used the primer pair ITS1 - (5'-CTTGGTCATTAGAGGAAGTAA-3') and ITS2 - (5'-GCTGC GTTCTTCATCGATGC-3') to amplify the fungal ITS-1 region by PCR. Each sample has an eight-base unique sequence. The amplifications were conducted using the initial DNA denaturation step at 95 °C for 2 min, followed by 25 cycles at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s and a final extension at 72 °C for 5 min. PCR reactions contained 4 μL of 5 × FastPfu Buffer, 0.8 μL of each primer (5 μmol/L), 2 μL of 2.5 mM dNTPs, 10 ng template DNA and 0.4 μL FastPfu Polymerase in a 20 μL final volume.

2.3. Illumina MiSeq sequencing and data processing

We extracted the amplicons from 2% agarose gels, used the Axy-Prep DNA Gel Extraction Kit to purified amplicons, and quantified using QuantiFluor™ according to the manufacturer's instructions. We pooled the purified amplicons in aliquots and sequenced paired-end (2 × 250) using an Illumina MiSeq platform according to the standard instructions. We analyzed the raw data by the NCBI Sequence Read Archive (SRA) database.

Raw files were quality-filtered and demultiplexed by QIIME (Caporaso et al., 2010) as the following standard: (a) The 300 bp reads were truncated at any site receiving an average quality score <20 over a 50 bp sliding window, and truncated reads <50 bp were discarded. (b) Ambiguous characters were removed to ensure exact barcode reads matched. (c) According to the overlap sequence, only the sequences which overlap longer than 10 bp were assembled. And the other unassembled reads were discarded.

We used UPARSE (Edgar et al., 2011) to cluster and assign the sequences to Operational Units (OTUs) with 97% similarity cutoff and we used UCHIME (Edgar, 2013) to identify and remove the chimeric sequences. Sequences were assigned to operational taxonomic units (OTUs) with a threshold of 97% pair-wise identity and then classified taxonomically using Ribosomal Database Project (RDP) classifiers (Broadhurst et al., 2012). The RDP classification assignments were randomly confirmed using BLASTN by comparing sequences of strains reported in the NCBI database. Rarefaction curves were plotted for each sample to ensure adequate coverage. To further fungal community diversity and relative abundance, the fungal was classified taxonomically at the phylum and genus level.

We calculated the Chao to estimate the richness, and Simpson index were calculated to estimated diversity of each soil sample. Coverage index was selected to calculate the percentage of the sequenced total species in each soil sample. Rarefaction analyses were performed in Analytic Rarefaction v.1.3 (Hunt Mountain Software, USA).

2.4. Root rot disease severity, disease index and ginseng growth rate of root weight analysis

The disease severity of the roots were rated after three successive seasons using a-to-f scale, where a showed no visible lesions on root, b showed lesions up to 0.9 mm in diameter and appeared brown, c showed lesions of 1–4.0 mm and appeared dark brown, d showed lesions of 4–7.0 mm and appeared black, e showed lesions coalesced with each other, and f showed fully rotten on root. The disease severity index and the root rot disease index were calculated with three replications and the growth rate of root weight was calculated with five replications.

The disease severity index (DSI) = $([Xa \times 1] + [Xb \times 2] + [Xc \times 3] + [Xd \times 4] + [Xe \times 5] + [Xf \times 6]) / (Xa + Xb + Xc + Xd + Xe + Xf)$, where Xa, Xb, Xc, Xd, Xe, and Xf represent the numbers of plants with rotting severity of a, b, c, d, e, and f, respectively (Rahman and Punja-Z, 2006).

Root rot disease index = Amount of infected plants/Total amount of plants \times 100%

Growth rate of root weight = $(\text{Root weight (3 seasons later)} - \text{root weight (original not planted)}) / \text{Root weight (original not planted)} \times 100\%$

2.5. Statistical analyses

Read numbers were used to calculate OTU frequencies for variance analyses (ANOVA). Differentiate significant differences were tested by Duncan's tests and one-way analysis of variance among the seven treatments. The significance level was chosen at alpha = 0.05.

3. Results

3.1. Effects of the PAs on sequence data, soil fungi taxonomic richness and diversity

Rarefaction curves for each of the seven soils (Fig. 1) were constructed to evaluate soil richness and diversity. Rarefaction

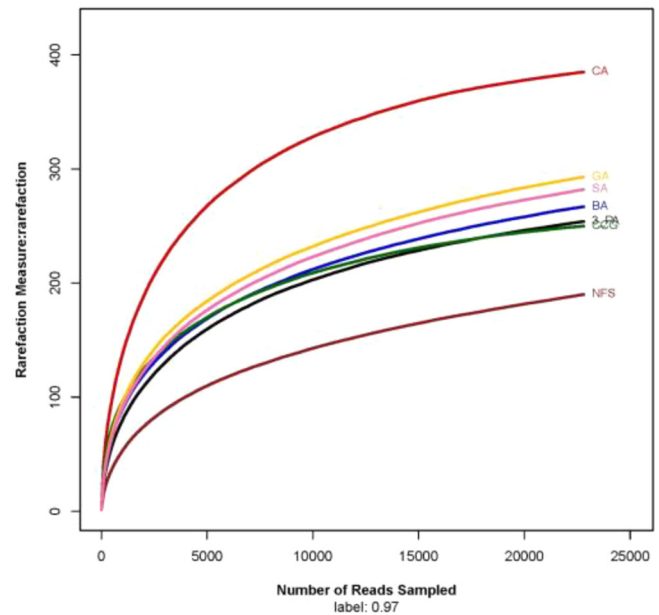


Fig. 1. Rarefaction curves at the 97% similarity level. Rarefaction curves showing the relationship between sampling intensity and the number of recovered OTUs from the different seven treatments. Sequences of the nuclear internal transcribed spacer region were grouped at 97% sequence identity.

analyses showed that the number of recorded OTUs tended to each plateau at 22,799 sequence reads. The lowest OTU numbers for the fungal ITS sequences were observed in the NFS sample. The curves were sufficient to show differences among treatments and suggested that PAs increased the fungal richness. Additionally, the sequencing coverage of the seven samples supported that the rarefaction curves achieved sufficient sequencing depths. The coverage ranged from 0.996 to 0.998 in all samples (Table 1).

A total of 201,617 high quality sequences from seven soils samples were obtained with a range between 23,004 to 35,277 sequences. Among these sequences, an average length of 252 bp optimized reads were detected by the MiSeq platform and these sequences were grouped into 197–389 OTUs at a 97% similarity level. We used Chao1 indices to evaluate species richness, both of that indicated that OTUs found in PAs-treated soil (except CA) were significantly higher than the CCG and the NFS soils. We observed a similar trend in the OTU variations in different samples. The number of OTUs in the CCG and NFS soils was 250 and 190, respectively, and these values were significantly lower than the PAs (except CA) in soil. The Simpson and Shannon indices were selected to indicate the species diversity. The diversity of OTUs found in PAs-treated soil (except CA) were higher than the NFS soil and the CCG soil (Table 1).

Table 1

Richness and diversity at the similarity level of 97% for fungi in seven soils. Different letters in each column indicate statistically significant differences at the 0.05 probability level according to the Duncan test.

Soils	OTUs	Chao	Simpson	Coverage
3-PA	254b	303c	0.1042d	0.997a
GA	293e	350d	0.0981cd	0.996a
SA	282d	342d	0.0912d	0.996a
BA	267c	328cd	0.0807b	0.997a
CCG	250b	268b	0.0572a	0.998a
CA	385f	413e	0.0576a	0.997a
NFS	190a	248a	0.3107e	0.997a

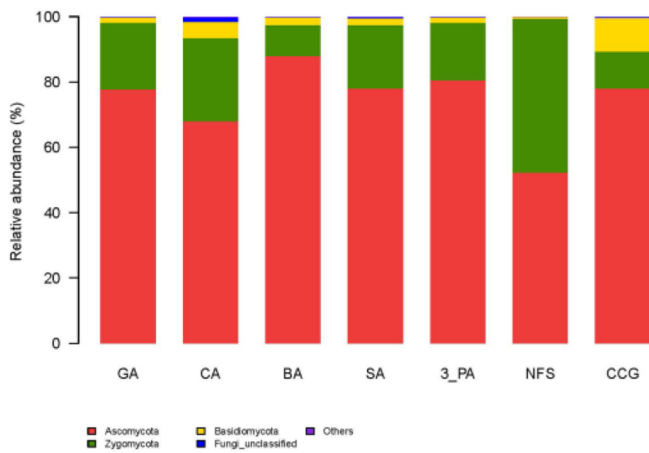


Fig. 2. Numerically dominant clades in the fungal communities at the phylum level. Relative abundances of the main fungal phyla in the soil of the seven treatments. The relative abundances of those fungal DNA sequences are shown that could be ascribed to a phylum of fungi, except for “Others”, which comprises all the rare remaining phyla.

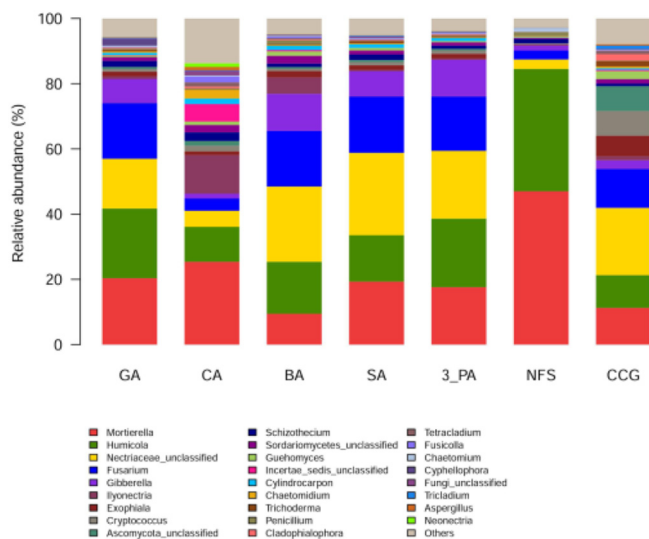


Fig. 3. Numerically dominant genus in the fungal communities at the class level. Relative abundances of the main fungal genus in the soil of the seven treatments. The relative abundances of those fungal DNA sequences are shown that could be ascribed to a genus of fungi, except for “Others”, which comprises all the rare remaining genus.

3.2. Effects of the PAs on soil fungi community structure and composition

There were 9 phyla, 23 classes, 60 orders, 116 families, and 206 genera in communities of the seven soils. At the phylum level, the sample community was dominated by Ascomycota. The distribution of Ascomycota in GA, CA, BA, SA, 3-PA, NFS, CCG samples was 77.7%, 68.0%, 87.9%, 78.1%, 80.5%, 52.3% and 78.0% respectively; for Zygomycota, 20.4%, 25.6%, 9.5%, 19.4%, 17.6%, 47.0% and 11.3% respectively. The rare phyla of all the soils were classified as Basidiomycota, Fungi unclassified, and others (Fig. 2).

For the class level, Mortierella, Humicola, Nectriaceae_unclassified, Fusarium, and Gibberella were the main classes (more than 50% in total) of the seven samples. Substantial differences were observed in community composition among these samples (Fig. 3). The NFS community had the greatest abundances of Mortierella and Humicola and the least abundances of Nectriaceae_unclassified, and Fusarium. The PAs (except BA) increased the abundances of Mortierella compared to the CCG sample. For the Nectriaceae_unclassified, Fusarium and Gibberella, CA decreased the abundances of these classes compared to the CCG sample.

Six clusters with highly supported were clustered together based on the Bray-Curtis distance (Fig. 4). Hierarchical clustering analysis revealed that the fungal communities in CCG and PAS-treated samples were similar. The community structures resulting from the Pas (3-PA, GA, SA, and BA)-treated soil were clustered together, but these groups were separated from the group of CA-treated. In addition, the group of 3-PA-treated displayed the largest differences from the NFS group.

Hierarchical clustering analysis of fungal communities based on Bray-Curtis distance indices of the rhizosphere soil amended with different seven treatments. The clustering of samples is based on Bray-Curtis distance indices calculated by OTUs at a distance of 0.03. The relative abundances of those fungal DNA sequences are shown that could be ascribed to a genus of fungi, except for “Others”, which comprises all the rare remaining genus.

3.3. Effects of the PAs on ginseng disease index, disease severity and growth rate

After three seasons of ginseng growth, the growth rate (as determined by root weight), disease indexes of root rot, and *Cylindrocarpon destructans* disease severity of the plants in different treatments were measured. (Table 2, Figs. 5 and 6) The growth rate of the NFS (82.48%) was significantly higher than the other treatments. Soils from CCG had higher growth rate than the PAS-treated (except CA) but lower than NFS. CA was different from

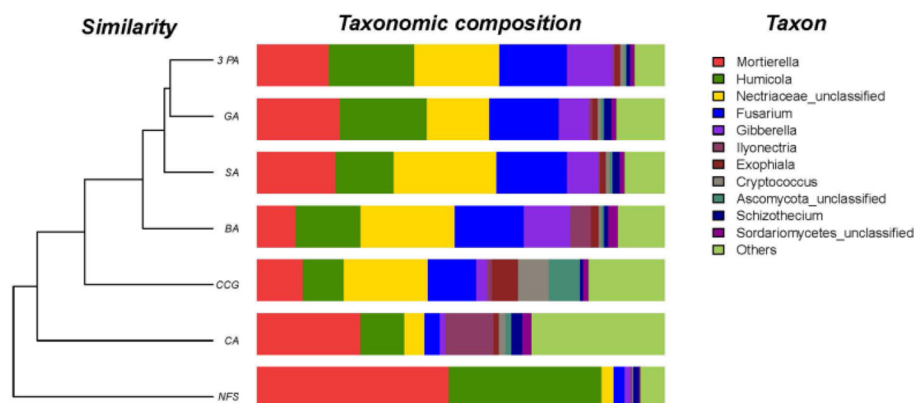


Fig. 4. Hierarchical clustering analysis in the fungal communities at the genus level.

Table 2
Disease severity of *Cylindrocarpon destructans* in seven treatments. Bars above the histogram represent the standard error of three replicates. Letters above the bars indicate a significant difference according to Duncan's multiple range test at $P < 0.05$ level.

NFS	CA	CCG	BA	SA	GA	3-PA
0.90a	2.20b	2.60c	4.00d	4.17de	4.23e	4.23e

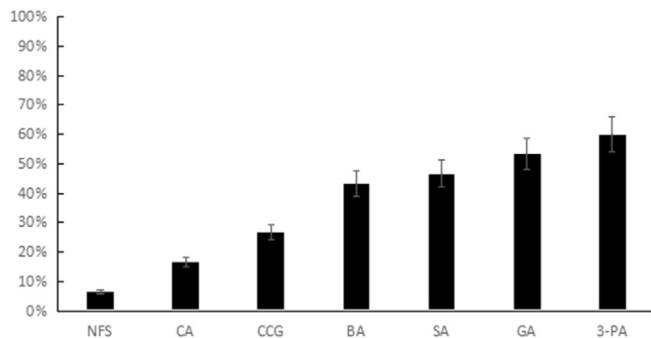


Fig. 5. Root rot disease indexes of seven treatments. Bars above the histogram represent the standard error of three replicates. Letters above the bars indicate a significant difference according to Duncan's multiple range test at $P < 0.05$ level.

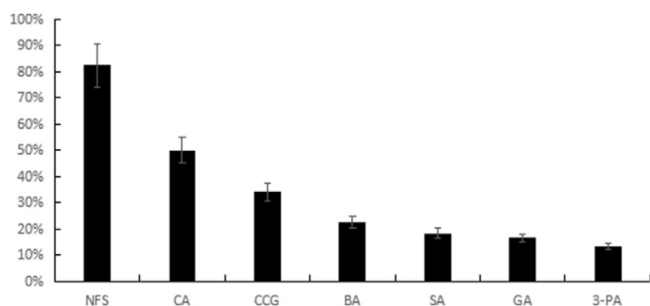


Fig. 6. Growth rate of root weight of seven treatments. Bars above the histogram represent the standard error of five replicates. Letters above the bars indicate a significant difference according to Duncan's multiple range test at $P < 0.05$ level.

the other 4 PAs. Root rot disease indexes of the NFS were significantly lower than other treatments. Root rot disease indexes of PAs-treated soil (except CA) were significantly higher than the index of CCG. Similarly, *Cylindrocarpon destructans* disease severity of NFS was lower than the other treatments. The severity of CCG was lower than the severity of PAs-treated (except CA) but higher than the severity of NFS.

4. Discussion

Microbial diversity and community composition significantly affect agricultural soil productivity, plant growth, and crop quality (Nayyar et al., 2009; Wu et al., 2011). Variation in the diversity and composition of the microbial community is believed to be related to changes in many factors (Derinkuyu et al., 2017; Mandava et al., 2017; Salameh et al., 2018; Scoppa et al., 2017). Such as cropping systems and root exudates (Bell et al., 2013; Yang et al., 2014). Continuous cropping system and root exudates such as the PAs affect soil microbial diversity and community composition, and thereby exerting significant negative impacts on soil productivity and health (Chen et al., 2014; Nayyar et al., 2009). In present study, the richness and diversity index analyses indicated that the PAs could enhance the fungi richness and decrease the fungi diversity under the new forest soil. Cluster analysis indicated that the PAs (except CA) changed the fungi structure compared to CCG and NFS

treatments, and these treatments behaved similarly on fungi community. We speculate the cinnamic acid is not the major PA accumulating from consecutive ginseng monoculturing (Banerjee et al., 2017; Kuscu et al., 2017; Messina et al., 2017; Singh et al., 2017).

Techniques of culture-dependent or culture-independent identification have been characterized by previous studies to confirm the fungi community composition. Recently, fungal community composition, abundance, and diversity have also been investigated by molecular techniques may underestimate diversity based on limited accessions in clone libraries. However, the molecular techniques lead to decreased taxa to identify the microbes based on clone libraries. For example, the fungi community in ginseng rhizosphere have been reported limitedly by PCR-DGGE (Xiao et al., 2016). The aim of present study was to investigate the effects of different PAs from ginseng rhizosphere on fungi structure and diversity in soil using the Illumina MiSeq platform. Our approach not only identified conventional fungi community taxa, but also revealed many rare fungi community taxa like *Tetracladium*, *Cladophialophora*, and *Fusicolla*. Additionally, a small number of "unclassified" sequences were identified in this study. This means that these soils contain some, as of yet, unidentified fungi.

As for the fungi community, the results of the present study agreed with previous studies at the phylum level but differed at the species level (Fig. 3). *Ascomycota*, *Zygomycota* and *Basidiomycota* were the dominant groups of the ginseng rhizosphere. Previous studies found *Phialocephala*, *Rhizomucor*, *Geomyces*, *Acremonium*, *Phacidium*, and *Hymenoscyphus* were the most common phyla in continuous cropping of ginseng rhizosphere (Xiao et al., 2016). In this study, members of other phyla, such as *Mortierella*, *Humicola*, *Nectriaceae*-unclassified, *Fusarium*, and *Gibberella*, have been described in the PAs treated soils of ginseng rhizosphere.

The relative abundances of OTUs, identified as *Mortierella* and *Humicola*, were significantly higher in NFS fields than the other fields. The abundances of *Mortierella* and *Humicola* in PAs fields were significantly higher than the CCG fields. Meanwhile, we observed that the abundances of *Nectriaceae*-unclassified, *Fusarium*, *Gibberella*, and *Cylindrocarpon* in PA fields were higher than the CCG fields, and the least abundance of these genes were in NFS fields. As we known, *Mortierella* and *Humicola* have not been reported as pathogens to ginseng previously. *Mortierella* is a widespread in the temperate zone. The disease incidence of *Phytophthora* blight, which is caused by *Phytophthora capsici* in peppers, and black spot on leaf, caused by *Alternaria brassicicola* in cabbage, were reduced by biocontrol fungi of *Humicola* (KO et al., 2011). *Phytophthora* and *Alternaria* also cause *Phytophthora cactorum* Schroet and *Alternaria* *Panax* in ginseng field (Zhao et al., 1993; Sun et al., 2014). The genus *Fusarium* comprises a lot of species in agricultural, environmental, and human health importance in soil. However, the *Fusarium* mainly results to pathogenicity towards a large number of plants, such as cucumber (Zhou and Wu, 2012), wheat (Dong et al., 2014), and cotton (Gaspar et al., 2014). *Gibberella* was an important soil-borne gene, which caused gibberella ear rot in maize and wheat scab. Additionally, *Gibberella* was the perfect stage of *Fusarium* (Vigier et al., 2001). We found that the distribution trends were similar between *Nectriaceae*-unclassified and *Cylindrocarpon destructans*, and the *Nectria*, which belong to *Nectriaceae*, was the perfect stage of *Cylindrocarpon destructans*. Therefore, *Nectriaceae*-unclassified, may be an abundant sexual morph of *Cylindrocarpon destructans*. In short, it may be that the increase of

soil-borne pathogenic fungi reduced the biotic suppression of soil-borne diseases by other non-pathogenic soil fungi with PAs treatment under consecutive ginseng monoculturing.

Ginseng root rot caused by *Fusarium* and *Cylindrocarpon* is the main cause for yield decline and poor growth of monocultured ginseng (Wang et al., 2016). Continuous cropping of other crops, such as peanut, pea or soybean, are also afflicted by *Fusarium* root rot elsewhere (Nayyar et al., 2009; Xu et al., 2012). The present study showed the numerous and abundant pathogenic fungal OTU belonged to Nectriaceae-unclassified, *Fusarium*, *Gibberella*, and *Cylindrocarpon*. These were the main causal agents of ginseng root rot in our study.

The other major ginseng pathogens, such as *Sclerotinia ginseng* Wang et Chen and *Rhizoctonia solani* were not found in this study. A possible reason may be that they are obligate parasites and thus, would predominate in plants but not necessarily in soil. The other possible reason may be that different pathogens infect plants at different growth stages. *Sclerotinia ginseng*, for example, primarily infects plants from April to May, but our sampling time was in October (Wang et al., 2016). The autotoxicity exduates in ginseng rhizosphere are very complex. PAs is a small group section of the exduates. The new metabolomics techniques might offer the potential to study the chemical differences in the autotoxicity exduates of ginseng (Floros et al., 2017). In this study, the influence of different treatments to the metabolomic composition of rhizosphere in ginseng was not researched. The relationship between metabolomic composition of autotoxicity exduates in ginseng rhizosphere and the microbial community is largely unknown. Finding out this relationship maybe contributed to remove the obstacle of continuous cropping in ginseng.

5. Conclusions

The present study showed that 579 fungal operational taxonomic units at 97% ITS sequence identity were found among 201,617 sequence reads derived from 18 separate soil samples. Members of the phylum *Ascomycota* dominated the soil fungal communities, and putative pathogens, such as *Fusarium*, *Gibberella* and *Nectriaceae_unclassified* which may include the abundant sexual morph of *Cylindrocarpon destructans*, showed higher relative abundances in the CCG fields. Compared to the CCG and NFS fields, PAs (except CA) enhanced the fungi richness and decreased fungi diversity. Cluster analysis indicated that the PAs (except CA) changed the fungi structure in a uniform way. PAs (except CA) stimulate root rot disease and enhance disease severity, restricting plant growth. In conclusion, the PAs (except CA) from ginseng rhizosphere enhance the fungi richness, decrease the fungi diversity, and change the fungi structure to increase fungal pathogen loads, which could explain the declined yield and quality of ginseng in consecutive monoculturing of ginseng.

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