

Microbial diversity and physicochemical properties in farmland soils amended by effective microorganisms and fulvic acid for cropping Asian ginseng

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Abstract

Demand for products made from the dry mass of *Asian ginseng* (*Panax ginseng*) is growing, but harvest is limited by fungal disease infection when ginseng is replanted in the same field. Rotated cropping with maize can cope with the replant limit, but it may take decades. We aimed to amend post-maize-cropping farmland soils for cultivating *Asian ginseng*, using effective microorganisms EMs and fulvic acid (FA) additives and detecting and comparing their effects on soil microbial diversity and physicochemical properties. Amendments promoted seedling survival and depressed disease-infection. Both EMs and FA increased the relative abundances of *Pseudomonas*, *Flavobacterium*, *Duganella*, and *Massilia* spp., but, decreased the relative abundances of *Fusarium* and *Sistotrema*. In addition, soil nutrient availability and properties that benefitted nutrient availabilities were promoted. In conclusion, amendments with EMs and FA improved the fertility of farmland soils, and the quality of *Asian ginseng*, and revealed the relationship between soil microbial diversity and physicochemical properties.

Keywords: Asian ginseng; effective microorganisms; farmland soil quality; fulvic acid; soil microbial activities

Introduction

Asian ginseng (*Panax ginseng* L., 1753), known as the king of herbs in East Asia due to its outstanding efficacy in pharmacological activity, is an important medicinal plant or natural tonic in China (Shin *et al.*, 2000; Soldati, 1999). Owing to the great commercial benefits of Asian ginseng extracts, the promotion of its dry mass products is highly desired by Asian ginseng planters and natural product managers. However, the replant failure generates a remarkable obstacle for continually cropping *Panax ginseng*, which has been attributed to accumulated toxic substances in replant sites' soils (Dong *et al.*, 2018). No solution was available to cope with

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the allelopathic inhibitory effects from replant cropping except for employing rotation cycles for decades (Dong *et al.*, 2018; Jiao *et al.*, 2019). An upgraded culturing protocol is needed for sustainable Asian ginseng cultivation with a shorter rotation cycle.

Soils for growing Asian ginseng require a desired soil quality, high microbial diversity (Xiao *et al.*, 2016; Zhao *et al.*, 2017; Tong *et al.*, 2021), and a high nutrition input (Beyaert, 2006; Kang *et al.*, 2016) for significant production and expected seedling growth. Remnant chemicals such as fertilizers and pesticides are commonly seen in farmland soils (Li *et al.*, 2015). This stimulates uncertainties in soil quality and microbial diversity and even cause failure of establishment, or impact growth and development of Asian ginseng stocks. One of the negative consequences on Asian ginseng is deteriorating diseases that occur in farmland soils from some combined effects of microbial communities and physicochemical properties (Dong *et al.*, 2018; Wang *et al.*, 2016). To improve farmland soils for optimizing field performance of Asian ginseng, adding amendments is suggested (Sun *et al.*, 2015). However, it is unclear how microbial communities and physicochemical properties respond to the amendment strategy for Asian ginseng cultivation. Information on the effects of different amendment strategies on the inner correlation between microbial diversity and physicochemical properties is scarce.

Deep composting is a general strategy to cope with the negative effects of substrates caused by unknown microbial communities and chemical remnants. Effective microorganisms (EMs) are an efficient compound microbial reagent that accelerates decomposition and transformation of organic matter in soils. Empirical practices reveal that additives with EMs can increase the number of soil microbes, activate soil enzymes, control pedosphere diseases, and promote plant growth (Borowiak *et al.*, 2021). Additionally, they contribute to amended soils in organic composting (Park, 2011), fruit and vegetable cultivation (Tommonaro *et al.*, 2021), and chemical degradation (Xing *et al.*, 2007; Kim *et al.*, 2011). These together suggest the potential of using additives with EMs to optimize farmland soils for Asian ginseng planting. To our knowledge, however, trials on farmland soils that were amended by EM additives for Asian ginseng cultivation are highly rare. The role and efficacy of EMs amendment in improving soil properties, enzyme activities, microbial diversity, nutrient absorption, and chemical reducing in *Asian ginseng* soils need to be further studied.

The other strategy to cope with farmland soils that have been negatively impacted is the optimization of soil structure by adjusting physicochemical properties to a desired level. Fulvic acid (FA) widely exists in nature as kinds of combined humic acid and water-soluble polymer (Gong *et al.*, 2020). FA contains more than 70 minerals and nucleic acids, soluble sugars, amino acids, peptides, phytochemical compounds, and vitamins (Malan, 2015). Most FA exist in an ionic form, suggesting a strong physiological activity with absorption, exchange, complexation and other functions (Marosz, 2009). Therefore, FA was widely used as an amendment for soils in agriculture, forestry, animal husbandry, and environmental protection (Aydin *et al.*, 2017). Amendment with FA can improve soil quality by promoting aggregate structure, nutrient utilization, and soil organic matter (SOM) accumulation. These will further provide a carbon (C) source for microbial growth and stabilizing soil temperature to enhance enzyme activity by adjusting soil pH and motivating microbial activities (Cheng *et al.*, 2013; Kotrocó *et al.*, 2014). Some bioorganic fertilizers were developed on the basis of FA-synthesized substrates, which were found to enhance hydrolyzation of alkali-hydrolysable nitrogen (N), available phosphorus (P), and soluble potassium (K) and activate enzymes, rhizosphere soil bacteria, and actinomycetes (Abdel-Baky *et al.*, 2019; Li *et al.*, 2021). Again, few studies have tested the application of FA amendment for Asian ginseng soils.

Increasing evidence indicates that soil microbes are an important and active component that closely relate to soil fertility and health (Hadar and Papadopoulou, 2012). The diversity and contents of microbial communities can directly reflect soil health and function (Visser and Parkinson, 1992). Soil microbes play an important role in soil formation (Pratscher *et al.*, 2011), development, material transformation (Jackson *et al.*, 2012), nutrient cycles (Kuzakov and Xu, 2013), organic matter transformation, pollutant degradation, and environmental purification (Cupples, 2005). The increase of beneficial microbes can promote nutrient uptake (Guiñazú *et al.*, 2010) and enhance resistance to disease (Cummings, 2009). Growth, development, and

productivity of plants interplay with soil microbial communities (Chaparro *et al.*, 2012). Soil amendment with additives will break the trade-off between plant and soil and form a new relationship between microbial diversity and physicochemical properties. Thus, one approach to meet Asian ginseng' cultivation target is to determine, in amended soils, microbial diversity and its relationship with physicochemical properties so soil amending regimes can be compared and evaluated.

In this study, farmland soils following maize rotation were amended with EMs and FA for cultivation of Asian ginseng stocks. Soil microbial composition and dominant population were quantified by high-throughput sequencing technology. Hence the relationship between soil microbial diversity and physicochemical properties can be detected. We hypothesized that: (i) farmland soils amended with EMs and FA will show a contrasting difference in composition and dominant population, with (ii) soil physicochemical properties significantly changed, and (iii) all these responses will synchronize as correlations between at least five pairs of variables in microbial diversity and physicochemical properties. Accordingly, it will be of great interest to further study the practice of amendments with EMs and FA as candidate strategies to improve farmland soils for better Asian ginseng production.

Materials and Methods

Site description

The experiment was conducted in a field of farmland that was prepared to cultivate *Asian ginseng*. The site was located in the eastern montane area. It had an average altitude of 672 m (43°41'-43°58' N, 128°10'-128°32' E) in Jilin Province, Northeast China. It has a temperate continental monsoon climate and four distinct seasons, with an average annual temperature of 2.6 °C, rainfall of 618 mm, relative humidity of 68%, and wind velocity of 2.7 m·s⁻¹. The land was rotated with maize before the commencement of this experiment, and the farmland was left fallow for one year.

Experimental design and farmland soil amendment

The study began on 20 April 2019. The farmland used had a total area of 500 m² (100 m × 5.0 m). It was divided into three plots, each with an area of 150 m² (100 m × 1.5 m), for Asian ginseng cultivation. Each plot was further divided into three operational blocks as three random replicates of sampling units. Each block had an area of 45 m² (30 m × 1.5 m) and a buffer (5.0 m × 1.5 m). Deep tillage was employed for all plots to a depth of 25 cm by a rotary cultivator before soil amendment. Adjacent cultivating areas were buffered by a lined land without any cropping. The first plot was used as a control (CK) without amendment. The second plot was designed to be amended by EMs (C1). EMs contained more than 100 billion live bacteria per gram (Organic-Biotech Inc., Luoyang, Henan, China). The third plot was amended by FA (C2) (Macklin Inc., Shanghai, China). Three weeks after the first rotary operation, additives of EMs and FA evenly rose to surface soil at a rate of 1.5 g·m⁻². In the second rotation, additives were given to soil 25 cm belowground to mix amendments with the soil. These practices were repeated once every four weeks for a total of three times up to late September 2019.

Asian ginseng seedling cultivation

Asian ginseng seeds were obtained from a seed source in Fusong County, Jilin Province, China. This was run as a ginseng orchard of National and Local Joint Engineering Research Center for Ginseng Breeding and Application (43°48' N, 125°24' E), Jilin Agricultural University, Changchun City, Northeast China. Seeds were collected on 10 August 2019, treated by the Hierarchic Optimization Method to facilitate seeding. Seeds were reserved in washed sands at room temperature for 30 days. On 25 March 2020, seeds were germinated at an average temperature of 24 °C with relative humidity ranging between 49% and 89%. On 5 May 2020, germinated embryos were transplanted to the experimental plots of CK, C1, and C2. Embryos were directly

sown to the blocks which were subsequently shaded by sunshades placed 1.5 m above the ground. Two-layer fibers were used to shade ginseng seedlings during the culture. The outer layer was black porous shading fiber, and the inner layer was yellow-rainproof-plastic fiber film. This manufacture can allow sunlight illumination at a transmittance of about 35%. During the experiment, temperature was 11.8/31.5 °C (night/day) and the relative humidity ranged between 49% and 89%. *Asian ginseng* seedlings were planted in a spacing of 15 cm × 15 cm for five months. Therefore, a total of 2,000 *Asian ginseng* seedlings were planted in a block and 6,000 in a plot.

Seedling sampling and determination

On 10 October 2020, the experiment was terminated, and seedlings were sampled from CK, C1, and C2 plots. Twenty-five seedlings were randomly sampled from a block, bulked as a sampling unit, and measured for survival rate, infected rate, stem growth (length and diameter), leaf variables (length, width, area, and chlorophyll concentration), and root growth (length, diameter, and weight).

Soil sampling

The soil type used in the experimental farmland was dark brown soil. On 25 April 2020, the soils were randomly sampled at a depth of 5-10 cm belowground from five points along an "s" shape across a block and mixed to a block unit. This way, three composite samples were collected in each experimental field for repetition. Consequently, nine samples were collected in three fields. Visible plant and animal residues were carefully removed from the fresh soil samples. An amount of 1.0 kg samples was left according to the quartering method, moved into the sterile sampling tubes, sealed, and transported to the laboratory. Some samples were air dried at indoor temperature then screened through 2 mm sieve. Half of the dried soil samples were used for determining physicochemical properties. The rest were sent to the laboratory at 0-4 °C and stored at ultralow temperature (-80 °C) for high-throughput sequencing analysis.

Soil physicochemical properties analyses

A total of 5.0 g soil sample was measured for the weight after air-drying and the dried weight after being oven-dried at 105 °C then measured for dried weight. Soil water content was calculated using the difference between dried and fresh soil weights. Soil organic matter was determined using the K₂Cr₂O₇ titration method. Hydrolyzed N was measured by the alkali hydrolysable method. Soil pH value was measured at a soil/water ratio of 1:2.5 (w/w) with a pH meter (PHSJ-6 L, INESA Scientific Instrument Inc., China). Electric conductance (EC) was measured at a soil/water ratio of 1:5 (w/w) with an EC meter (DDSJ-319 L, INESA Scientific Instrument Inc., China). Concentrations of available N, P, K, NH₄⁺-N and NO₃⁻-N was determined spectrophotometrically following the methods used in previous studies (Triplett Jr. and Van Doren Jr., 1969; Wei *et al.*, 2014; Zhu *et al.*, 2016; Wei *et al.*, 2017; Wei *et al.*, 2019). Total N and P concentrations were determined through colorimeter analysis following digestion with H₂SO₄-H₂O₂. Total K concentration was determined using inductively coupled argon plasma-optical emission spectroscopy. Available P concentrations were extracted with the HCl/NH₄F method. Samples were determined colorimetrically using ascorbic acid molybdate on a continuous flow autoanalyzer (Autoanalyzer III, Bran and Luebbe, Germany). Available K concentration was determined through NH₄OAc extraction. K chloride extraction indophenol blue colorimetry was used for ammonia acetate extraction and Inductively Coupled Plasma Plus Optical Emission Spectrometry (ICP-OES) was used for determination of metal ions.

Soil urease activity (Guan, 1986) was determined through phenol sodium colorimetry, and enzyme activity was expressed by the milligram of NH₄⁺-N in an amount of 1.0 g soils after reacting in 24 h. Sucrase activity was determined by 3,5-Dinitrosalicylic acid colorimetry, and activity was expressed by the milligram of glucose in 1.0 g soil after 24 h. Catalase activity was determined by potassium permanganate titration. Copper blue oxidase was determined through 2,2-diazo-di (3-ethyl-benzothiazole-6-sulfonic acid) diamine salt (ABTS)

colorimetry. Enzyme activity can produce free radicals through the decomposition of substrate ABTS by laccase, and activity can be calculated by the increase rate of free radicals.

High throughput sequencing

According to the E.Z.N.A.® DNA soil Kit (OmegaBio-tek, Norcross, GA, USA), total DNA extraction was carried out. DNA concentration and purity were detected with a NanoDrop2000 device, and the quality of DNA extraction was detected by 1% agarose gel electrophoresis. DNA was amplified by the PCR using the primer set 338F (5'-ACTCCTACGGGAGGCAGCAG-3'), 806R (5'-GGACTACHVGGGTWTCTAAT-3') for the V3-V4 regions of 16S rRNAs, or ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3'), and ITS1R (5'-GCTGCGTT CTTCATCGATGC-3') for the fungal ITS2 sequences (Inceoglu *et al.*, 2010). The amplification was processed as follows: pre-denaturation at 95 °C for 3 min, 27 cycles (denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s), and extension at 72 °C for 10 min (PCR: ABIGeneAmp® 9700). The amplification system was 20 µL with 4 µL 5 × FastPfu buffer, 2 µL 2.5 mM dNTPs, 0.8 µL primer (5 µM), 0.4 µL FastPfu polymerase, and 10 ng DNA template.

The PCR gel product was recovered by 2% agarose gel, purified by AxyPrep DNA Gel Extraction Kit (AxygenBiosciences, UnionCity, CA, USA), eluted by elution, and detected by 2% agarose gel electrophoresis. Quantifluor™-st (Promega, USA) was used for detection and quantification. According to the standard operating procedures of Illumina Miseq platform (Illumina, SanDiego, USA), the purified amplification fragments were constructed into the library of PE2×300. Library construction followed four steps: first, connect the "Y" shaped connector; second, use magnetic beads to screen and remove the self-linked segments of the connector; third, use PCR amplification to enrich the library templates; finally, produce single strand DNA fragments with sodium hydroxide denaturation.

Bioinformatics analysis

The original sequences were controlled by TRIMMOMATIC software and spliced by FLASH software: (i) set up a 50 bp window, if the average quality in the window was less than 20, cut off all the sequences at the back end of the base from the front end of the window, and remove the sequences with a length less than 50 bp after quality control; (ii) spliced the sequences at both ends according to the overlapping base, where the maximum overlap fell in a range between overlaps during splicing, the mismatch ratio was 0.2 and the length should be more than 10 bp; (iii) according to barcodes and primers at both ends, sequences were divided into each sample. However, barcodes needed to match accurately. The primers allowed the mismatch of two bases, and removed the sequences with fuzzy bases. The UPARSE software (version 7.1) (UPARSE software, 2021) was used to cluster the sequences with operational taxonomic units (OTUs) according to 97% similarity, and to remove single sequences and mosaics in the process of clustering (Edgar, 2013). The RDP classifier was used to annotate the species classification of each sequence (Wang *et al.*, 2007). The representative sequence was assigned to the Silva database (SSU123) (Pruesse *et al.*, 2007). The threshold of comparison was set to 70% to obtain the classification information of bacterial community. Bacteria were classified according to an order of phylum, class, order, family and genus.

Statistical analysis

One-way analysis of variance (ANOVA) was used for the comparison of difference among three types of amendment treatments of CK, C1, and C2. Physicochemical properties of amended soils, soil enzyme activity, and seedling variables were analyzed using one-way ANOVA. Data were tested for normality and homogeneous variances. All of our data passed these two tests and no data transformation was needed. IBM SPSS Statistics 24.0 software (SPSS Inc., Chicago, IL, USA) was used to compare the above-mentioned variables, microbial diversity indices, and the relative abundances of soil microbial communities. The microbial relative abundances of CK, C1 and C2 were different at the genus level. They were then confirmed by a principle coordinate analysis, which was conducted on the basis of weighted UniFrac distance. Analysis of

similarity was used to depict differences in the compositions of the microbial community. The heatmap was generated using the plots package in R (version 2.15.3) to compare the top 45 bacterial and fungal genera in soil samples of CK, C1 and C2. Statistical analysis of soil factors and the top 20 microbial genera was used to screen soil variables with $P > 0.05$. The selected environmental factors, such as pH, SOM, iron, soil catalase, laccase, urease, and invertase, were used for correlation analysis. These correlation analyses were also performed to assess the relationships among soil properties, plant growth, and soil microbial community. Biodiversity for soil microbial community was assessed by Shannon index (Wang *et al.*, 2018).

Results

Soil physicochemical properties

Significant difference was found as responses in variables of SOM, pH, $\text{NH}_4^+\text{-N}$, available P, Ca, Mg, Fe, and Zn under different amendment treatments (Table 1). The concentrations of $\text{NH}_4^+\text{-N}$, available P, Ca, Mg, and Zn were higher in the soils amended by FA than that in CK, but soil pH results were reversed.

Table 1. Soil physicochemical properties in farmland soils amended by nothing (CK), EMs (C1), and FA (C2)

Parameters	CK	C1	C2
Available Mg ($\text{mg}\cdot\text{kg}^{-1}$)	198.6 ± 0.90c	290.3 ± 0.08a	226.8 ± 0.50b
Available Ca ($\text{mg}\cdot\text{kg}^{-1}$)	1556.9 ± 12.10c	2412.7 ± 7.00a	1748.9 ± 8.30b
Available Mn ($\text{mg}\cdot\text{kg}^{-1}$)	56.3 ± 0.41b	56.8 ± 0.31b	63.3 ± 0.20a
Available Fe ($\text{mg}\cdot\text{kg}^{-1}$)	295.8 ± 2.03c	346.0 ± 1.22b	381.6 ± 1.70a
Available Zn ($\text{mg}\cdot\text{kg}^{-1}$)	9.4 ± 0.01b	13.5 ± 0.01a	15.6 ± 0.04a
Available P ($\text{mg}\cdot\text{kg}^{-1}$)	65.2 ± 0.69b	126.4 ± 0.03a	178.6 ± 5.50a
Soil $\text{NH}_4^+\text{-N}$ ($\text{mg}\cdot\text{kg}^{-1}$)	0.7 ± 0.29c	4.2 ± 0.05b	10.1 ± 0.20a
SOC ($\text{g}\cdot\text{kg}^{-1}$)	24.2 ± 0.92a	27.9 ± 0.61a	30.6 ± 4.40a
Soil pH value	5.0 ± 0.05b	5.3 ± 0.16a	4.6 ± 0.08c
Soil moisture content (%)	18.7 ± 0.01c	20.7 ± 0.01b	21.0 ± 0.01a
Soil bulk density ($\text{g}\cdot\text{cm}^{-3}$)	0.95 ± 0.12a	0.94 ± 0.10a	0.93 ± 0.10a
Soil urease activity ($\text{U}\cdot\text{g}^{-1}$)	414.7 ± 17.30c	642.7 ± 14.7b	922.0 ± 18.4a
Soil sucrase activity ($\text{U}\cdot\text{g}^{-1}$)	10.9 ± 1.07c	42.0 ± 5.72a	19.6 ± 2.80b
Soil catalase activity ($\text{U}\cdot\text{g}^{-1}$)	20.7 ± 0.16b	22.2 ± 0.19a	16.4 ± 0.30c
Soil laccase activity ($\text{U}\cdot\text{g}^{-1}$)	38.8 ± 1.52b	46.0 ± 0.91a	47.1 ± 1.80a

Note: The letters indicate the tested with shortest significant ranges at the $P = 0.05$ of different treatments. Different lowercase letters indicate that there were significant differences at the $P < 0.05$ level

Also, as it is shown in Table 1, concentrations of soil $\text{NH}_4^+\text{-N}$ in the C1 treatment was 4.181 $\text{mg}\cdot\text{kg}^{-1}$, which was 5.65 times higher than that in CK. Concentration of available P was 1.94 times higher than that in CK. Concentrations of available Ca, Mg, Zn, and SOM in the C1 treatment increased by 2.413 $\text{g}\cdot\text{kg}^{-1}$, 290.270 $\text{mg}\cdot\text{kg}^{-1}$, 13.480 $\text{mg}\cdot\text{kg}^{-1}$, and 27.930 $\text{g}\cdot\text{kg}^{-1}$, respectively, from those in CK. Soil bulk density was 0.94 $\text{g}\cdot\text{cm}^{-3}$ in the C2 treatment, which had no difference from that in the C1 treatment and CK. Concentration of $\text{NH}_4^+\text{-N}$ increased significantly in the C2 treatment compared to the C1 and CK. The available P concentration in the C2 treatment was 178.63 $\text{mg}\cdot\text{kg}^{-1}$, which was 2.74 times higher than that in CK. Concentrations of available Ca, Mg, Zn, and SOM in the C2 treatment were 1.75 $\text{g}\cdot\text{kg}^{-1}$, 226.8 $\text{mg}\cdot\text{kg}^{-1}$, 15.55 $\text{mg}\cdot\text{kg}^{-1}$, 30.59 $\text{g}\cdot\text{kg}^{-1}$, respectively, and soil bulk density was 0.97 $\text{g}\cdot\text{cm}^{-3}$.

In Table 1, it was also found that amendments of EMs and FA had significant effects on soil enzyme activities compared to CK. Enzyme activities of soil urease, laccase, catalase, and invertase were activated in the

C1 treatment compared to CK. Soil urease and laccase activities, however, were significantly increased in the C2 treatment. Enzyme activities of soil catalase and invertase were lower in the C2 treatment than in CK.

Soil microbial compositions

After taking the same size from each sample to the same sequencing depth (61562 reads per sample) and clustering, a total of 298,014 OTUs with 97% identity were obtained, and the number of OTUs per sample fell in a range between 23,181 and 47,569 as illustrated in Figure 1. Subs index of OTUs increased to be near an upper limit, and the goodness of fit reached 99.46%. This suggests that the detection rate of the microbial communities in soil samples was increased with sampling numbers up to saturation. In addition, the current sequencing amount covered most species in these samples. The curves of Shannon index tended to be flat, and the amount of sequencing data was large enough to reflect the vast majority of microbial information in these soils.

As it is shown in Table 2, the diversity indices of soil bacterial community were different in CK, C1 and C2. Shannon index was lower in the C2 treatment than that in the C1 treatment and CK. In contrast, Simpson index was higher in the C2 treatment than that in the C1 treatment and CK. Both Ace and Chao indexes were lower in the C2 than that in C1 and CK. Results of these two different algorithms verified or corroborated each other.

Table 2. Diversity index of soil bacterial community in the experimental farmland (CK, C1 and C2)

Parameters	Shannon	Simpson	Ace	Chao
CK	6.20a	0.0060b	2103.7a	2081.2a
C1	6.48a	0.0042b	2294.5a	2292.2a
C2	5.36b	0.0194a	1773.0b	1791.7b

Note: The letters indicate the tested with Shortest Significant ranges at the P = 0.05 of different treatments. Different letters denote a significant difference at the P < 0.05 level.

As was listed in Table 3, the diversity indices of soil fungal community were different among CK, C1 and C2. Compared to CK, Shannon index was lower in the C1 treatment, with no significant difference between C2 and CK. Simpson index was higher in C1 compared to that in CK and C2. The diversity of soil fungal community was highest in CK and lowest in C1. Similar findings were reflected by the two algorithms, which were verified by each other. The Shannon index was unchanged in C2 from that in CK, but both were higher than that in C1. In contrast, Simpson index was higher in C1 than in CK and C2. The indexes of Ace and Chao did not show significant difference among the three types of amendment treatments.

In this study, non-metric multidimensional scaling (NMDS) analysis method was used on the diversity of soil microbial communities. As seen in Figure 2, there was a significant difference in soil microbial communities between CK and the C1 and C2 treatments. In bacterial and fungal compositions, soil microbial community was affected mainly by EMs and FA additives.

Table 3. Diversity index of soil fungal community in the experimental farmland (CK, C1 and C2)

Parameters	Shannon	Simpson	Ace	Chao
CK	2.76a	0.1769b	397.5a	390.7a
C1	1.64b	0.4818a	367.3a	347.2a
C2	2.40a	0.1944b	405.6a	408.7a

Note: The letters indicate the tested with Shortest Significant ranges at the P = 0.05 of different treatments. Different letters denote a significant difference at the P < 0.05 level.

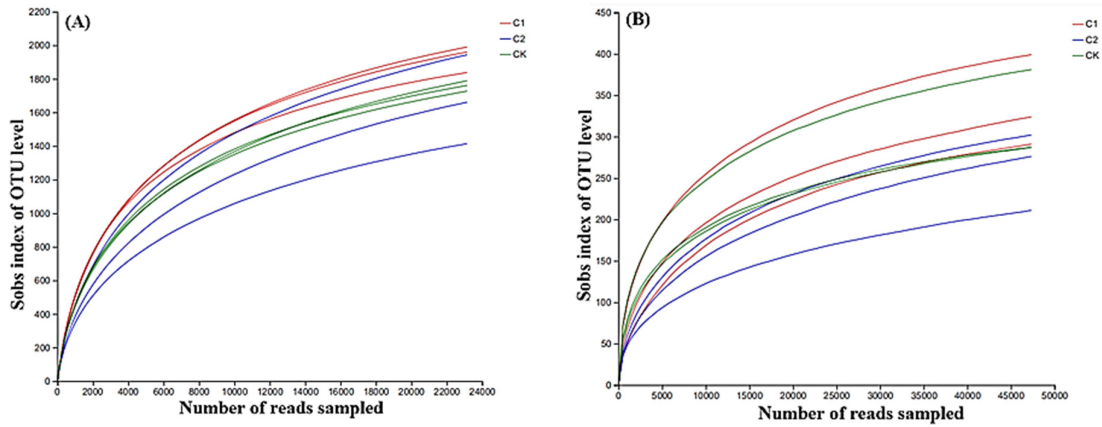


Figure 1. Images of dilution curve of soil microbial communities in CK, C1 and C2, the curves of different colours represent different treatments of farmland soil samples, bacterial community (A) and fungal community (B), respectively

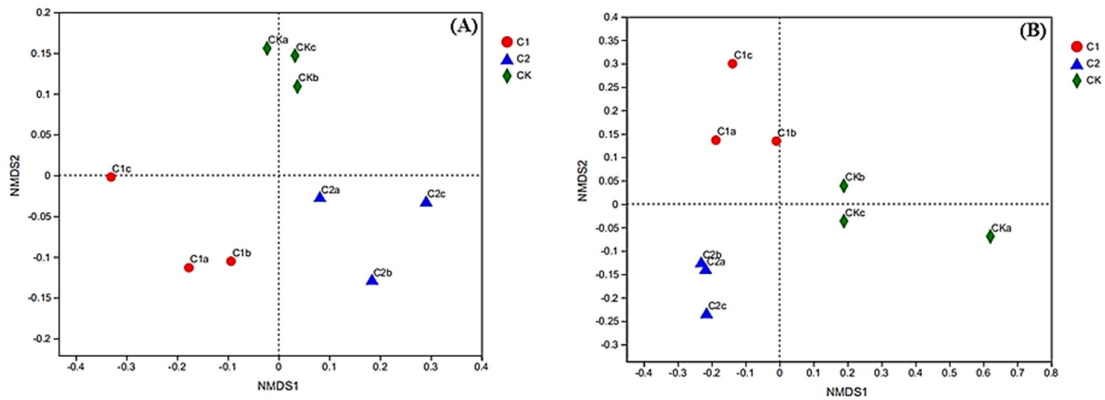


Figure 2. Images of composition of soil microbial communities in CK, C1 and C2, different colours and shapes represent different treatments of farmland soil samples, bacterial community (A) and fungal community (B), respectively

As was described in Figure 3, the heatmap showed the composition analysis of bacteria community in CK, C1, and C2 treatments. A total of forty-five genera were detected, and the black colour in the diagram represents the relative abundance of a certain genus. The relative abundances of *norank_p_Saccharibacteria*, *Sphingomonas*, and *norank_f_Acidobacteriaceae_Subgroup_1* accounted for 10%, 9%, and 7%, respectively, while those of *norank_o_Gaiellales*, *Rhodanobacter*, *Rhizomicrobium*, *norank_f_ODP1230B8.23*, and *Bradyrhizobium* accounted for 4% in total. The relative abundances of *Pseudomonas*, *Roseiflexus*, *norank_f_Anaerolineaceae*, *norank_o_Subgroup_7*, *RB41*, *Pedobacte*, *Massilia*, *Flavobacterium*, *Duganella*, and other bacteria were lower in the bacteria community of CK. Compared to CK, the relative abundances of *Mizugakiibacter*, *Mizugakiibacter*, *Pseudomonas*, *RB41*, *Duganella*, and others were significantly higher in C1, with the increases of *Massilia*, *norank_c_Acidobacteria*, *norank_o_Subgroup_7*. The relative abundance of *Duganella* was significantly lower, and those of *Rhodanobacter*, *Granulicella*, *norank_f_Ktedonobacteraceae* were slightly lower. Compared with C1, the relative abundances of *Acachidicoccus*, *Pseudomonas*, *Mizugakiibacter* and *Arthrobacter*, as well as *Rhodanobater*, were significantly higher in C2.

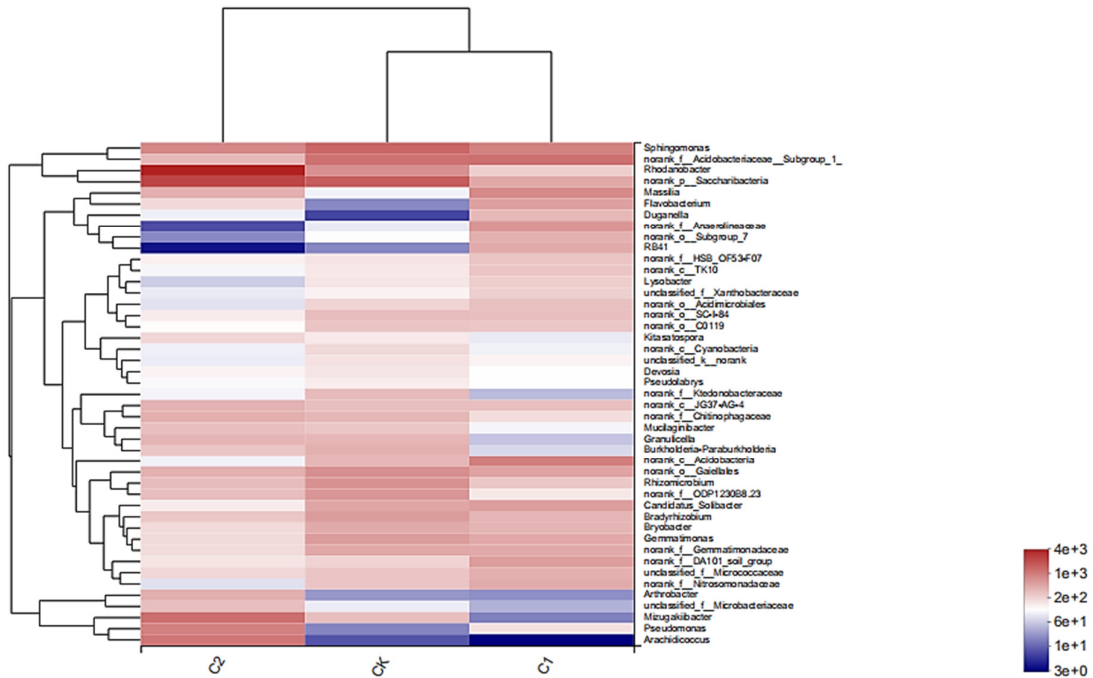


Figure 3. The relative abundances of forty-five bacterial genera in CK, C1 and C2 amendments. Deeper red colours indicate higher levels of relative abundances, deeper blue colours label lower levels of relative abundances.

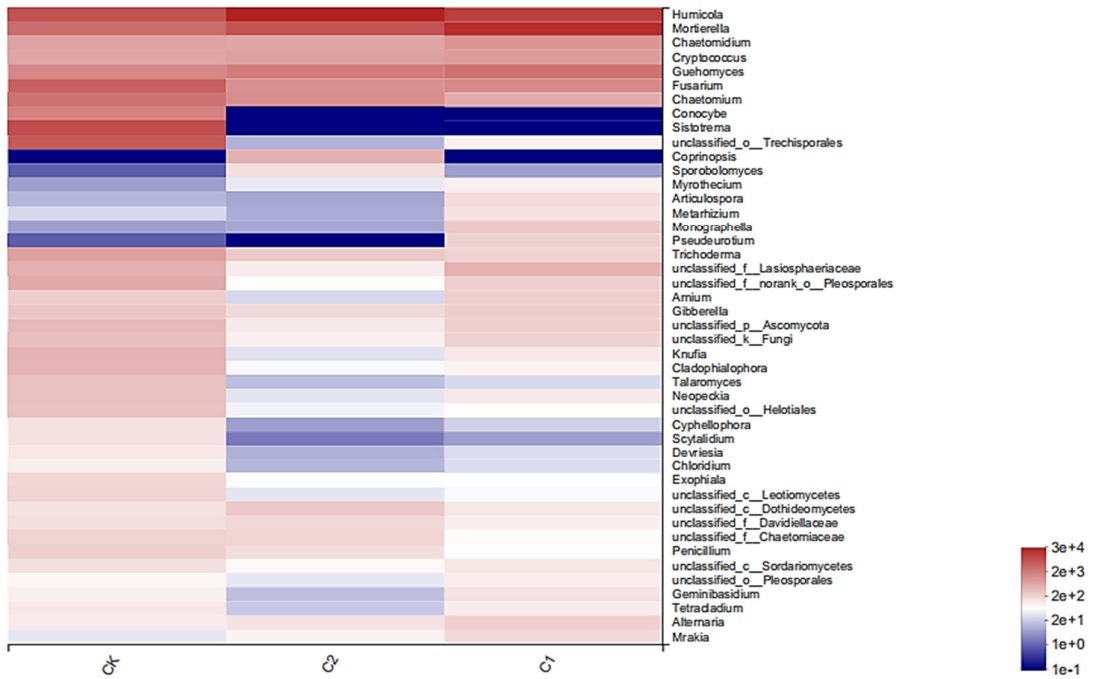


Figure 4. The relative abundances of forty-five fungal genera in CK, C1 and C2 types of amendments. Deeper red colours indicate higher levels of relative abundances; deeper blue colours label lower levels of relative abundances.

The heatmap (Figure 4) showed the compositions of fungi community in CK, C1, and C2. A total of forty bacteria genera were detected, in which the color block represents the relative abundance of a certain genus. The relative abundance of *Sistotrema* in CK was 19%, while those of *Humicola*, *unclassified_o_Trechisporales*, *Fusarium*, *Mortierella*, *Chaetomium*, *Guehomyces*, and *Conocybe* were 16%, 13%, 11%, 8%, 6%, 4%, and 4%, respectively. The relative abundances of *Coprinopsis*, *Sporobolomyces*, *Myrothecium*, *Articlesopora*, *Metarhizium*, *Monographella*, *Pseudoeurotium*, and *Mrakia* were lower compared to CK. The relative abundances of *Chaetomium*, *Knupia*, *Cladophialophora*, *Neopeckia*, *Talaromyces*, *Sistotrema*, *Fusarium*, and *Conocybe* decreased significantly, while those of *Humiola*, *Chaetomium* and *Pseudoeurotium* increased. Compared with C1, the relative abundance of *Pseudoeurotium* did not change, except for an insignificant decrease. The relative abundances of *Fusarium*, *Chaetomium*, *Knutia* and other fungal community decreased more significantly, while *Humiola* and *Chaetomium* increased more.

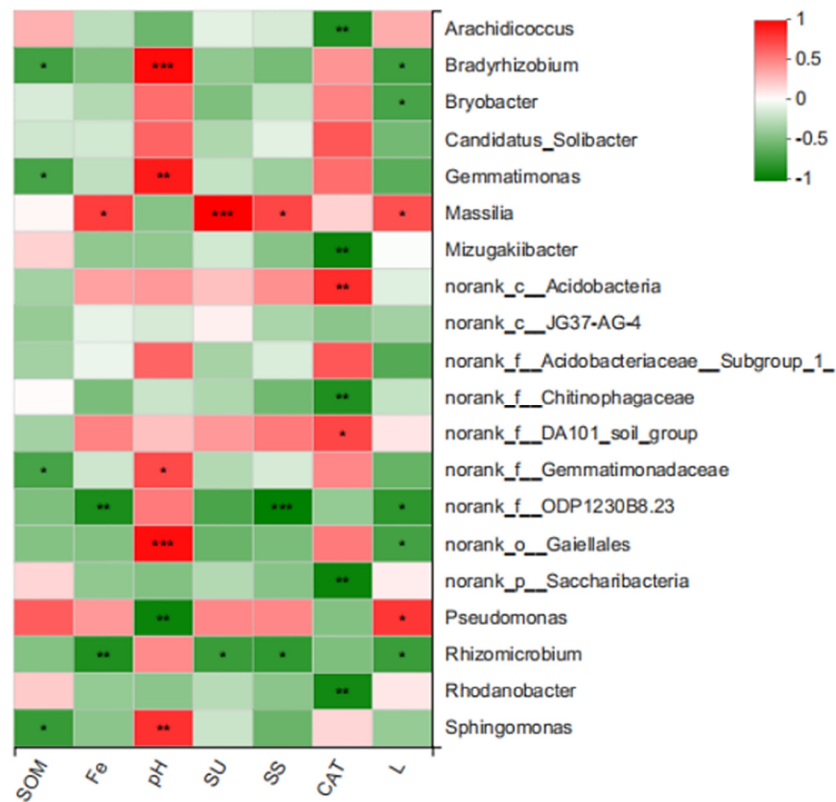


Figure 5. The correlation heatmap between bacteria of the top twenty genera and soil physiochemical properties

The cluster tree of species and environmental factors can be selected. The correlation R value and P value are obtained by calculation, the R value is shown in different colours in the figure, if the P value is less than 0.05, it is marked with * and the legend on the right is the colour range of different R values, e.g. the symbol * represents the P value at $0.01 < P \leq 0.05$ level, the symbol ** represents the P value at $0.001 < P \leq 0.01$ level, the symbol *** represents the P value at $P \leq 0.001$ level.

SOM: soil organic matter, SU: soil urease, SS: soil sucrose, CAT: soil catalase, L: soil laccase

Figure 5 showed the relationship between soil physicochemical properties and bacterial community compositions across amended farmland soils. SOM negatively correlated with the relative abundances of *Bradyrhizobium*, *gemmatimonas*, *norank_f_gemmatimonadaceae*; iron concentration negatively correlated with *Massilia* community abundance and positively correlated with *norank_f_ODP1230B8.23* community

abundance; pH value negatively correlated with *Pseudonas* community abundance and positively correlated with community abundances of *Bradyrhizobium*, *Gemmatimonas*, *Sphingomonas*, *norank_o_Gaiellales*, and *norank_f_Gemmatimonadaceae*. However, soil urease negatively correlated with *Bradyrhizobium* and positively correlated with *Massilia*. Soil invertase positively correlated with *Massilia*, negatively correlated with *Bradyrhizobium*, and had a significant negative correlation with *norank_f_ODP1230B8.23*. In addition, a negative correlation was found between soil catalase and community abundances of *Archidicoccus*, *Mizugakiibacter*, *Rhodanobacter*, and *norank_f_Chitinophagaceae*. A positive correlation was found between soil catalase and community abundances of *norank_c_Acidobacteria* and *norank_f_DA101*. Soil laccase had a negative relationship with community abundances of *Bradyrhizobium*, *Bryobacter*, *Rhizomicrobium*, *norank_o_Gaiellales*, and *norank_f_ODP1230B8.23*. Soil laccase also had a positive correlation with community abundances of *Massilia* and *Pseudomonas*.

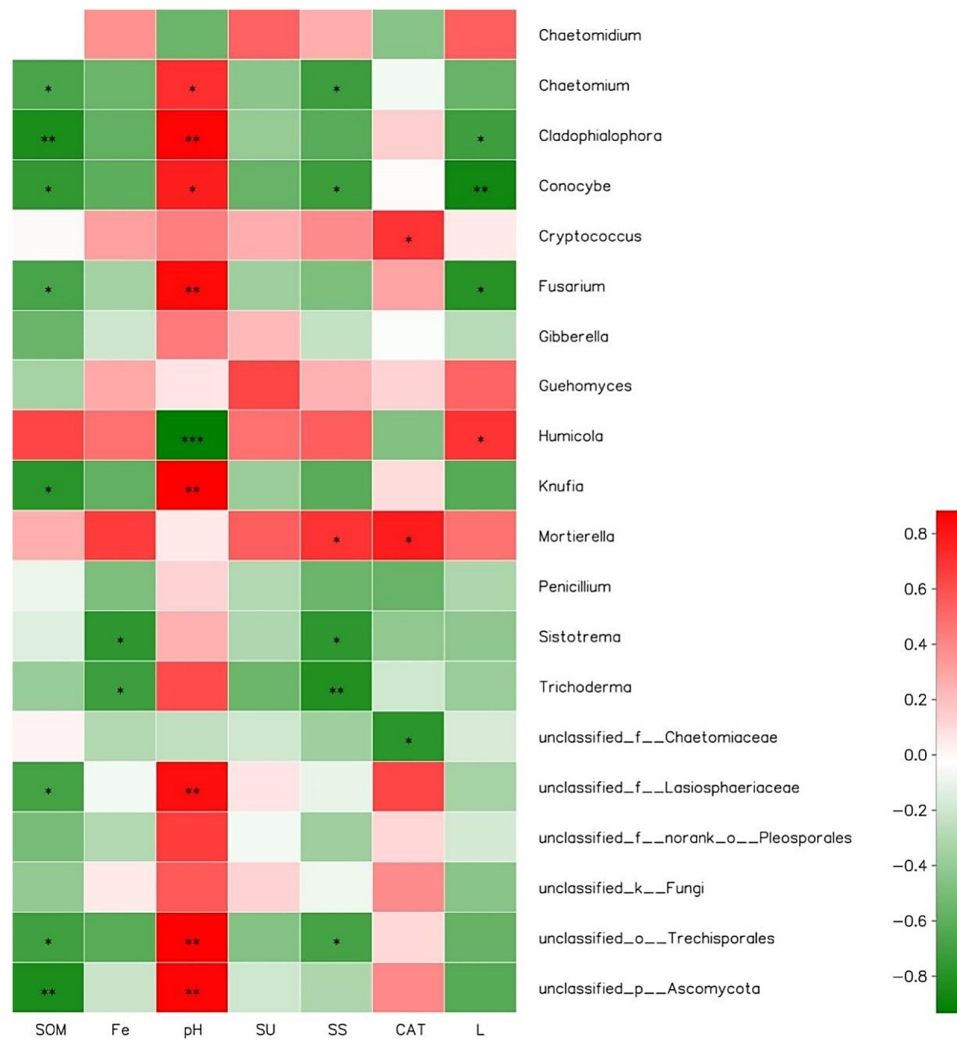


Figure 6. The correlation heatmap between fungi of the top twenty genera and environmental factors. The cluster tree of species and environmental factors can be selected. The correlation R value and P value are obtained by calculation, the R value is shown in different colours in the figure, if the P value is less than 0.05, it is marked with * and the legend on the right is the colour range of different R values, e.g. the symbol * represents the P value at $0.01 < P \leq 0.05$ level, the symbol ** represents the P value at $0.001 < P \leq 0.01$ level, the symbol *** represents the P value at $P \leq 0.001$ level.

SOM: soil organic matter, SU: soil urease, SS: soil sucrose, CAT: soil catalase, L: soil laccase

As shown in Figure 6, SOM negatively correlated with community abundances of *Chaetomium*, *Conocybe*, *Fusarium*, *Knufia*, *unclassified_f_Lasiosphaeriaceae*, and *unclassified_o_Trechisporales*, and with community abundances of *Cladophialophora* and *unclassified_p_Ascomycota*. Iron concentration negatively correlated with community abundances of *Sistotrema* and *Trichoderma*. Soil pH value positively correlated with community abundances of *Chaetomium*, *conocybe*, *Cladophialophora*, *Fusarium*, *Knufia*, *unclassified_f_Lasiosphaeriaceae*, *unclassified_o_Trechisporales*, and *unclassified_p_Ascomycota*, and negatively correlated with *Humicola* community abundance. In addition, soil invertase positively correlated with *Trichoderma* community abundance and negatively correlated with community abundances of *Chaetomium*, *Conocybe*, *Sistotrema*, and *unclassified_o_Trechisporales*. Soil catalase positively correlated with community abundances of *Cryptococcus* and *Mortierella*. It negatively correlated with *unclassified_f_Chaetomiaceae* community abundance. Soil laccase positively correlated with community abundances of *Cladophialophora*, *Humicolaand*, and *Fusarium* and negatively correlated with *Conocybe* community abundance.

Performance of Asian ginseng seedlings

As it is shown in Table 4, variables of seedling growth, as well as morphologies in root, stem, leaves, and leaf chlorophyll concentration, all improved in the C1 and C2 treatments compared to CK. In addition, disease rate of Asian ginseng seedlings decreased in soils with added amendments compared to that in the control. Survival rate was increased by soil amendments.

Table 4. Growth, chlorophyll concentration, and disease and survival rates in *Asian ginseng* seedlings subjected to farmland soil amendments by EMs (C1) and FA (C2) with an unamended control (CK)

Parameters	CK	C1	C2
Root length (cm)	7.37±0.03c	8.98±0.01b	9.17±0.02a
Root diameter (mm)	4.47±0.01c	5.27±0.03a	5.29±0.02b
Root weight (g)	0.37±0.02c	0.59±0.01a	0.58±0.01b
Stem length (cm)	8.68±0.02c	9.57±0.03b	9.65±0.01a
Stem diameter (mm)	0.95±0.01b	1.12±0.02a	1.13±0.02a
Leaf length (cm)	2.87±0.03c	3.33±0.02a	3.32±0.01b
Leaf width (cm)	1.75±0.02c	1.95±0.01b	1.97±0.02a
Leaf area (cm ²)	3.55±0.03b	4.45±0.03a	4.56±0.02a
Chlorophyll	29.69±0.02c	32.76±0.03b	33.43±0.22a
Seedling disease rate (%)	8.71±0.01a	1.55±0.02c	1.87±0.03b
Seedling survival rate (%)	75.22±0.03b	87.85±0.02a	89.16±0.01a

Note: The significance of differences between different treatments is indicated by lowercase letters ($P < 0.05$). Values followed by the same letter are not statistically different according to the independent t-test at $P < 0.05$.

Discussion

Soil microbes are the most active part, and an important part of material metabolism cycles (Bending *et al.*, 2000; Wang *et al.*, 2018). They not only participate in various metabolism activities as executor to shape SOM transformation (Prescott and Vesterdal, 2021), but also transform and hydrolyse biological residues and organic matters. These together promote the increase of soil nutrients. Moreover, the metabolisms of soil microbes are the main source of soil enzyme, and the correlation of nutrient transformation in soil enzymes. Therefore, it was speculated that soil microbes played an important, indirect role in the increase of soil fertility and ecosystem, and their diversity and activities were closely related to soil physicochemical properties. In this study, the modifiers of FA and EMs were added to farmland soils in order to investigate the response of soil microbes and their relationships with physicochemical properties. Overall results showed that bacterial and

fungal communities obviously changed in amended farmland soils. Soil microbes and physicochemical properties interplayed to promote change in soil quality.

Soil enzymes are the products of the decomposition of animal and plant residues, plant root exudates, and soil microbial metabolism. They are a kind of substance with biochemical catalytic activities and participate in many important biochemical processes in soils. These processes closely relate to the release and storage of various nutrients, the formation and development of humus in soil, and the soil structure and physicochemical properties. They are involved in the whole process of soil genesis and development, as well as the formation and evolution of soil fertility. Therefore, soil enzyme activity directly reflects changes in soil structures and compositions. The larger the index of soil enzyme is, the stronger its activity is and the stronger the soil fertility is. These results were consistent with the significant increase of soil nutrients, which also indicated that EMs and fulvic acid had significant effects on soil structures and fertility improved farmland soil.

Amendment with EMs had different effects on soil nutrients and enzyme activities. This variation of changes resulted from increases in concentrations of Ca, P, and N, activities of soil urease and sucrase, and soil pH value. These results showed that the EMs additive can improve soil physicochemical properties, available N and P, and the activities of soil enzymes through the physiological activities of microbes due to EMs containing phosphate solubilizing bacteria and nitrogen fixing bacteria (Higa and Wididana, 1991). Adding FA to the farmland soils was more effective than EMs in promoting soil quality with increasing concentrations of available P, hydrolysed N, and SOM, and reducing pH value. No changes in soil moisture content and bulk density were observed in this study. However, it was found that soil pH value and concentrations of SOM, iron, sucrase, laccase and catalase activities were closely related to the diversity of soil bacterial and fungal communities. These results suggested that organic matters that were chelated with iron were decomposed by soil bacteria and fungi at higher pH, which did not depend on additional water absorption (Smith and Paul, 1990).

It was also found that diversity of beneficial bacterial community had increased after farmland soils were amended. Parts of original components in bacterial community had changed, such as *Pseudomonas*, *Flavobacterium*, *Duganella* and *Massilia*. Other beneficial bacterial community with extremely low original relative abundances had significantly increased after the amendment. This was extremely significance to the changes of soil physicochemical properties and the strengthening of biological control of plant diseases. *Pseudomonas* is a kind of bacteria that plays an important role in biological control. The secondary metabolites of *Pseudomonas* can dissolve the soil Al (Saleh *et al.*, 2019), which is one of the greatest factors that cause *Asian ginseng* red skin disease (Liu *et al.*, 2014). The reduction of soil Al concentration possibly reduced the occurrence of *Asian ginseng* red skin disease. These results suggested that *Pseudomonas* had a great inhibitory effect on a variety of soil borne plant disease bacteria (Nagel *et al.*, 2012; Iglesias *et al.*, 2018; Aiello *et al.*, 2019).

The existence of *Flavobacterium* spp. plays an important role in soil microbes and plant growth (Soltani *et al.*, 2010). Results in the study revealed that *Flavobacterium* spp. increased concentrations of C and N in soils amended with EMs and FA. These changes promote the proliferation of soil microbes and plant growth (Aiello *et al.*, 2019; Herrera *et al.*, 2019). *Duganella* spp. are an important genus of *Fusarium* resistant bacteria, which can effectively reduce plant diseases caused by a variety of *Fusarium* spp. (Haack *et al.*, 2016). *Massilia* spp. is a kind of promising genus of bacteria in improving the soil farmland of cultivated *Asian ginseng*, and that also has an excellent ability of dissolving P fractions (Zheng *et al.*, 2017). This can increase the available P concentration, and produce dimethyl disulfide which is expected to replace methyl bromide fumigant (Feng *et al.*, 2016). Dimethyldisulfide has a significant effect on controlling soil pathogenic fungi and nematodes, while reducing the levels of pathogenic bacteria, soil fumigant, and pollution. In this study, EMs and FA changed the diversity of soil bacterial community in different degree. After adding FA to the farmland soils, the relative abundances of communities, such as *Achidicoccus*, *Pseudomonas*, *Mizugakiibacter*, and *Arthropcharacters* spp., as well as the relative abundance of *Rhodanobacter*, all increased accordingly. The enrichment of *Rhodanobater* spp. can improve the denitrification ability of soil (Green *et al.*, 2012), strengthen the supply of soil microbes and plant N element, and promote the growth of plant and the increment of soil microbes.

The analysis of soil fungal diversity showed that farmland soils amended with EMs and FA could reduce the diversity of harmful fungal community. After soil amendment, parts of the structures and compositions of the original microbes were changed significantly. Furthermore, the relative abundances of harmful fungi, such as *Sistotrema* spp. and *Fusarium* spp., reduced due to EMs additions. This accounted for the decline in soil borne diseases (Higa and Wididana, 1991). *Fusarium* spp. are a common plant pathogen, which can cause a variety of crop diseases (Chen *et al.*, 1995; Haack *et al.*, 2016). The increase in the relative abundance of *Duganella* spp. was likely due to soil amendment. This inhibited the propagation of *Fusarium* spp. Meanwhile, *Sistotrema* spp. have a bacteriostatic effect, and mycelia have antagonistic effects on *Bacillus Subtilis* and six other kinds of microbes (Haack *et al.*, 2016). The decrease of its relative abundance was beneficial to the growth of bacteria and the increase of soil fertility. In this study, the community abundances of *Fusarium*, *Chaetomium*, *Knupia*, and other fungal community decreased significantly, and those for *Humicola* and *Chaetomium* increased in amended soils compared to controlled soils. According to the correlation analysis of soil fungi and physiochemical properties, community abundances of *Fusarium*, *Chaetomium* and *Knunia* spp. had a significant positive correlation with soil pH, but, *Humicola* and *Chaetomium* had a significant negative correlation with soil pH. Accordingly, the changes of fungous community were caused by fulvic acid reducing the soil pH.

Soil pH is an important soil chemical factor affecting the diversity of soil bacteria (Landesman *et al.*, 2014). When soil pH was lower than 6.5, a significant negative correlation between soil bacterial diversity and soil pH will occur (Cho *et al.*, 2016). In the present study, the amended soils were slightly acidic, which were prone to enable the co-existence of multiple bacteria genera. The beneficial bacteria, such as *Bradyrhizobium*, *Gemmatimonas*, and *Sphingomonas* spp., had a significant positive correlation with pH values in amended soils. Soil pH may not directly change the structures and compositions of bacterial community, but it may impose a force that results in overall changes in the soil environment. Several soil properties were directly or indirectly affected by the change in pH value. These soil variables included, but were not limited to, concentrations of available nutrients, cation exchange, SOM, and factors that will also change the diversity of soil bacterial community (Lauber *et al.*, 2009). Besides, soil compositions had a significant influence on microbial communities (Girvan *et al.*, 2003), of which pH was thought to exert primary domination on the compositions of soil bacterial community (Landesman *et al.*, 2014). Therefore, diversity of soil bacterial community can be regulated by adjusting pH in agricultural production.

Diversity of soil fungal community was affected by environmental factors. According to the results of correlation analysis, soil pH was an important factor affecting fungal diversity. There was a significant positive correlation between various fungi, such as *Fusarium*, and pH, which indicated that fungi were more suitable for survival in acidic soil, and acidic soil would increase fungi reproduction. The results also showed that *Fusarium*, *Conocybe*, *Cladophilophora*, and other fungi had pathogenic effects on plants with a positive correlation with SOM, soil pH, and laccase activity. These soil variables played the same role to depress infection by fungi on *Asian ginseng*. Some fungi having pathogenic effects may have some physiological connections to some extent and the same response to a certain factor. Consequently, some variables can be adjusted to control the occurrence of soil pathogenic fungi in agronomy production.

Conclusions

In this study, we found that amendments with EMs and FA have unique effects on microbial structures and physiochemical properties in farmland soils planting Asian ginseng. The relative abundances of several communities of beneficial bacteria were changed by amendments, such as *Pseudomonas*, *Flavobacterium*, *Duganella*, and *Massilia* spp. Meanwhile, the relative abundances of pathogenic fungi, such as *Fusarium* and *Sistotrema* spp., were decreased by amendments. Changes in soil physiochemical properties and microbial community structure did not vary between treatments of amendments by EMs vs FA additives. Hence, two

types of amendments had a comparable impact on modifying microbial activities but varied in specific functions. EMs can increase beneficial bacteria diversity and inhibit the rate of fungal disease, while FA can enhance soil structure and availability. Overall, the use of EMs and FA as additives to amend maize-rotated soils can be an available approach for replanting Asian ginseng by improving biotic and abiotic properties in farmland soils. The combination of these two additives together may further result in a better outcome, but confirmative results need more work to be identified.

Authors' Contributions

Study design and conceptualization: Y.X., C.L. and Y.L.; Project administration: Y.L. and Y.X.; Investigation: C.L. and J.B.; Methodology: Y.X. and L.Z.; Resources: H.Z. and Y.C.; Data curation: C.L. and J.B.; Formal analysis: C.L. and Y.C.; Funding acquisition: Y.L. and Y.X.; Software: H.Z. and Y.C.; Supervision: Y.X.; Validation: Y.X. and C.L.; Visualization: Y.X. and L.Z.; Writing - original draft: C.L. and H.Z.; Writing - review and editing: Y.L.

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

- Abdel-Baky YR, Abouziena HF, Amin AA, Rashad El-Sh M, Abd El-Strar AM (2019). Improve quality and productivity of some faba bean cultivars with foliar application of fulvic acid. *Bulletin of the National Research Centre* 43(1):2. <https://doi.org/10.1186/s42269-018-0040-3>
- Aiello D, Restuccia C, Stefani E, Vitale A, Cirvilleri G (2019). Postharvest biocontrol ability of *Pseudomonas synxantha* against *Monilinia fructicola* and *Monilinia fructigena* on stone fruit. *Postharvest Biology and Technology* 149:83-89. <https://doi.org/10.1016/j.postharvbio.2018.11.020>
- Aydin SK, Dalgic S, Karaman M, Kirlangic OF, Yildirim H (2017). Effects of fulvic acid on different cancer cell lines. *Proceedings* 1(10):1031. <https://doi.org/10.3390/proceedings1101031>
- Bending GD, Putland C, Rayns F (2000). Changes in microbial community metabolism and labile organic matter fractions as early indicators of the impact of management on soil biological quality. *Biology and Fertility of Soils* 31(1):78-84. <https://doi.org/10.1007/s003740050627>
- Beyaert RP (2006). Influence of nitrogen fertilization on the growth and yield of North American ginseng. *Journal of Herbs, Spices and Medicinal Plants* 11(4):65-80. https://doi.org/10.1300/J044v11n04_08
- Borowiak K, Wolna-Maruwka A, Niewiadomska A, Budka A, Schroeter-Zakrzewska A, Stasik R (2021). The effects of various doses and types of effective microorganism applications on microbial and enzyme activity of medium and the photosynthetic activity of scarlet sage. *Agronomy* 11(3):603.
- Chaparro JM, Sheflin AM, Manter DK, Vivanco JM (2012). Manipulating the soil microbiome to increase soil health and plant fertility. *Biology and Fertility of Soils* 48(5):489-499. <https://doi.org/10.1007/s00374-012-0691-4>

- Chen C, Bauske EM, Musson G, Rodriguezkabana R, Kloepper JW (1995). Biological control of *Fusarium* wilt on cotton by use of endophytic bacteria. *Biological Control* 5(1):83-91. <https://doi.org/10.1006/bcon.1995.1009>
- Cheng F, Peng X, Zhao P, Yuan J, Zhong C, Cheng Y, Cui C, Zhang S (2013). Soil microbial biomass, basal respiration and enzyme activity of main forest types in the Qinling Mountains. *PloS One* 8(6):e67353. <https://doi.org/10.1371/journal.pone.0067353>
- Cho S-J, Kim M-H, Lee Y-O (2016). Effect of pH on soil bacterial diversity. *Journal of Ecology and Environment* 40(1):10. <https://doi.org/10.1186/s41610-016-0004-1>
- Cummings SP (2009). The application of plant growth promoting rhizobacteria (PGPR) in low input and organic cultivation of graminaceous crops; potential and problems. *Environmental Biotechnology* 5:43-50.
- Cupples AM (2005). Principles and Applications of Soil Microbiology. Second Edition. *Journal of Environmental Quality* 34(2):731-732. <https://doi.org/10.2134/jeq2005.0731dup>
- Dong L, Xu J, Li Y, Fang H, Niu W, Li X, Zhang Y, Ding W, Chen S (2018). Manipulation of microbial community in the rhizosphere alleviates the replanting issues in *Panax ginseng*. *Soil Biology and Biochemistry* 125:64-74. <https://doi.org/10.1016/j.soilbio.2018.06.028>
- Dong L, Xu J, Zhang L, Cheng R, Wei G, Su H, Yang J, Qian J, Xu R, Chen S (2018). Rhizospheric microbial communities are driven by *Panax ginseng* at different growth stages and biocontrol bacteria alleviates replanting mortality. *Acta Pharmaceutica Sinica B* 8(2):272-282. <https://doi.org/10.1016/j.apsb.2017.12.011>
- Dong LL, Xu J, Li Y, Fang HL, Niu WH, Li XW, Zhang YJ, Ding WL, Chen SL (2018). Manipulation of microbial community in the rhizosphere alleviates the replanting issues in *Panax ginseng*. *Soil Biology and Biochemistry* 125:64-74. <https://doi.org/10.1016/j.soilbio.2018.06.028>
- Edgar RC (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods* 10(10):996. <https://doi.org/10.1038/nmeth.2604>
- Feng GD, Yang SZ, Li HP, Zhu HH (2016). *Massilia putida* sp nov., a dimethyl disulfide-producing bacterium isolated from wolfram mine tailing. *International Journal of Systematic and Evolutionary Microbiology* 66:50-55. <https://doi.org/10.1099/ijsem.0.000670>
- Girvan MS, Bullimore J, Pretty JN, Osborn AM, Ball AS (2003). Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. *Applied and Environmental Microbiology* 69(3):1800-1809. <https://doi.org/10.1128/aem.69.3.1800-1809.2003>
- Gong G-Q, Zhao Y-F, Zhang Y-j, Deng B, Liu W-X, Wang M, Yuan X, Xu L-W (2020). Establishment of a molecular structure model for classified products of coal-based fulvic acid. *Fuel* 267:117210. <https://doi.org/10.1016/j.fuel.2020.117210>
- Green SJ, Prakash O, Jasrotia P, Overholt WA, Cardenas E, Hubbard D, ... Kostka JE (2012). Denitrifying bacteria from the genus *Rhodanobacter* dominate bacterial communities in the highly contaminated subsurface of a nuclear legacy waste site. *Applied and Environmental Microbiology* 78(4):1039-1047. <https://doi.org/10.1128/aem.06435-11>
- Guan YS (1986). Soil enzyme and its research methods. Beijing, China: Agricultural Press of China.
- Guiñazú LB, Andrés JA, Del Papa MF, Pistorio M, Rosas SB (2010). Response of alfalfa (*Medicago sativa* L.) to single and mixed inoculation with phosphate-solubilizing bacteria and *Sinorhizobium meliloti*. *Biology and Fertility of Soils* 46(2):185-190. <https://doi.org/10.1007/s00374-009-0408-5>
- Haack FS, Poehlein A, Kroger C, Voigt CA, Piepenbring M, Bode HB, Daniel R, Schafer W, Streit WR (2016). Molecular keys to the *Janthinobacterium* and *Duganella* spp. interaction with the plant pathogen *Fusarium graminearum*. *Frontiers in Microbiology* 7:1668. <https://doi.org/10.3389/fmicb.2016.01668>
- Hadar Y, Papadopoulou KK (2012). Suppressive composts: microbial ecology links between abiotic environments and healthy plants. In: Van Alfen NK, Leach JE, Lindow S (Eds). *Annual Review of Phytopathology*, pp 133-153.
- Herrera LM, Brana V, Fraguas LF, Castro-Sowinski S (2019). Characterization of the cellulase-secretome produced by the Antarctic bacterium *Flavobacterium* sp. AUG42. *Microbiological Research* 223:13-21. <https://doi.org/10.1016/j.micres.2019.03.009>
- Higa T, Wididana GN (1991). The concept and theories of effective microorganisms. In: Parr JF, Hornic SB, Whitman CE (Eds). *Proceedings of the First International Conference on Kyusei Nature Farming*. US Department of Agriculture, pp 118-124.
- Iglesias MB, Lopez ML, Echeverria G, Vinas I, Zudaire L, Abadias M (2018). Evaluation of biocontrol capacity of *Pseudomonas graminis* CPA-7 against foodborne pathogens on fresh-cut pear and its effect on fruit volatile compounds. *Food Microbiology* 76:226-236. <https://doi.org/10.1016/j.fm.2018.04.007>

- Inceoglu O, Hoogwout EF, Hill P, van Elsas JD (2010). Effect of DNA Extraction Method On The Apparent Microbial Diversity Of Soil. *Applied and Environmental Microbiology* 76(10):3378-3382. <https://doi.org/10.1128/aem.02715-09>
- Jackson LE, Bowles TM, Hodson AK, Lazcano C (2012). Soil microbial-root and microbial-rhizosphere processes to increase nitrogen availability and retention in agroecosystems. *Current Opinion in Environmental Sustainability* 4(5):517-522. <https://doi.org/10.1016/j.cosust.2012.08.003>
- Jiao X-L, Zhang X-S, Lu X-H, Qin R, Bi Y-M, Gao W-W (2019). Effects of maize rotation on the physicochemical properties and microbial communities of American ginseng cultivated soil. *Scientific Reports* 9(1):8615. <https://doi.org/10.1038/s41598-019-44530-7>
- Kang H, Hwang YG, Lee TG, Jin CR, Cho CH, Jeong HY, Kim DO (2016). Use of gold nanoparticle fertilizer enhances the ginsenoside contents and anti-inflammatory effects of red ginseng. *Journal of Microbiology and Biotechnology* 26(10):1668-1674. <https://doi.org/10.4014/jmb.1604.04034>
- Kim Y-K, Hong S-J, Jee H-J, Shim C-K, Park J-H, Han E-J, An N-H, Yoo J-H (2011). Population dynamics of effective microorganisms in microbial pesticides and environmental-friendly organic materials according to storing period and temperature. *The Korean Journal of Pesticide Science* 15(1):55-60.
- Kotroczo Z, Veres Z, Fekete I, Krakomperger Z, Tóth JA, Lajtha K, Tóthmérész B (2014). Soil enzyme activity in response to long-term organic matter manipulation. *Soil Biology and Biochemistry* 70:237-243. <https://doi.org/10.1016/j.soilbio.2013.12.028>
- Kuzyakov Y, Xu XL (2013). Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. *New Phytologist* 198(3):656-669. <https://doi.org/10.1111/nph.12235>
- Landesman WJ, Nelson DM, Fitzpatrick MC (2014). Soil properties and tree species drive beta-diversity of soil bacterial communities. *Soil Biology and Biochemistry* 76:201-209. <https://doi.org/10.1016/j.soilbio.2014.05.025>
- Lauber CL, Hamady M, Knight R, Fierer N (2009). Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology* 75(15):5111-5120. <https://doi.org/10.1128/aem.00335-09>
- Li Z, Liu Z, Zhang M, Chen Q, Zheng L, Li YC, Sun L (2021). The combined application of controlled-release urea and fulvic acid improved the soil nutrient supply and maize yield. *Archives of Agronomy and Soil Science* 67(5):633-646. [10.1080/03650340.2020.1742326](https://doi.org/10.1080/03650340.2020.1742326)
- Li ZB, Zheng DJ, Tian YQ, Che XQ, Zhou RJ, Fu JF (2015). Effects of pesticide-fertilizer on soil microbial population and physicochemical properties in *Panax ginseng* continuous cropping soil. *Journal of Jilin Agricultural University* 37(1):73-76 (in Chinese with English abstract).
- Liu X, Yang ZM, Gao LL, Xiang WY, Zhang B, Xie ZL, You JF (2014). Comparison of the characteristics of artificial ginseng bed soil in relation to the incidence of ginseng red skin disease. *Experimental Agriculture* 50(1):59-71. <https://doi.org/10.1017/s0014479713000367>
- Malan C (2015). Review: humic and fulvic acids. A practical approach. In: *Sustainable Soil Management Symposium*. Cape Town, South Africa, Agrilibrum Publisher.
- Marosz AJD (2009). Effect of fulvic and humic organic acids and calcium on growth and chlorophyll content of tree species grown under salt stress. *Dendrobiology* 62:47-53.
- Nagel K, Schneemann I, Kajahn I, Labes A, Wiese J, Imhoff JF (2012). Beneficial effects of 2,4-diacetylphloroglucinol-producing pseudomonads on the marine alga *Saccharina latissima*. *Aquatic Microbial Ecology* 67(3):239-249. <https://doi.org/10.3354/ame01595>
- Park K (2011). Composting of food waste and mixed poultry manure inoculated with effective microorganisms. *Engineering in Agriculture, Environment and Food* 4(4):106-111. <https://doi.org/10.11165/eaef.4.106>
- Pratscher J, Dumont MG, Conrad R (2011). Ammonia oxidation coupled to CO₂ fixation by archaea and bacteria in an agricultural soil. *Proceedings of the National Academy of Sciences* 108(10):4170-4175. <https://doi.org/10.1073/pnas.1010981108>
- Prescott CE, Vesterdal L (2021). Decomposition and transformations along the continuum from litter to soil organic matter in forest soils. *Forest Ecology and Management* 498:119522. <https://doi.org/10.1016/j.foreco.2021.119522>
- Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig WG, Peplies J, Glockner FO (2007). SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research* 35(21):7188-7196. <https://doi.org/10.1093/nar/gkm864>

- Saleh DK, Abdollahi H, Noaparast M, Nosratabad AF, Tuovinen OH (2019). Dissolution of Al from metakaolin with carboxylic acids produced by *Aspergillus niger*, *Penicillium bilaji*, *Pseudomonas putida*, and *Pseudomonas korensis*. *Hydrometallurgy* 186:235-243. <https://doi.org/10.1016/j.hydromet.2019.03.014>
- Shin HR, Kim JY, Yun TK, Morgan G, Vainio H (2000). The cancer-preventive potential of *Panax ginseng*: a review of human and experimental evidence. *Cancer Causes and Control* 11(6):565-576. <https://doi.org/10.1023/A:1008980200583>
- Smith JL, Paul EA (1990). The significance of soil microbial biomass estimations. In: Blollag JM, Stotzky G (Eds). *Soil Biochemistry*. 1st Edition. Taylor and Francis, Boca Raton, FL, USA, Routledge.
- Soldati F (1999). *Panax ginseng*: Standardization and Biological Activity. In: *Biologically Active Natural Products*. CRC Press.
- Soltani AA, Khavazi K, Rahmani HA, Omidvari M, Dahaji PA, Mirhoseyni H (2010). Plant growth promoting characteristics in some *Flavobacterium* spp. isolated from soils of Iran. *The Journal of Agricultural Science* 2:106-115.
- Sun H, Wang QX, Zhang YY, Yang Z, Xu CL (2015). Integrated evaluation of soil fertility of *Panax ginseng* under different cultivation modes. *Journal of Jilin Agricultural University* 37(3):323-331 (in Chinese with English abstract).
- Tommonaro G, Abbamondi GR, Nicolaus B, Poli A, D'Angelo C, Iodice C, De Prisco R (2021). Productivity and nutritional trait improvements of different tomatoes cultivated with effective microorganisms' technology. *Agriculture* 11(2):112. <https://doi.org/10.3390/agriculture11020112>
- Tong A-Z, Liu W, Liu Q, Xia G-Q, Zhu J-Y (2021). Diversity and composition of the *Panax ginseng* rhizosphere microbiome in various cultivation modes and ages. *BMC Microbiology* 21(1):18. <https://doi.org/10.1186/s12866-020-02081-2>
- Triplett Jr. GB, Van Doren Jr. DM (1969). Nitrogen, phosphorus, and potassium fertilization of non-tilled maize. *Agronomy Journal* 61(4):637-639. <https://doi.org/10.2134/agronj1969.00021962006100040047x>
- UPARSE software (2021). UPARSE OTU clustering. <http://drive5.com/uparse/>
- Visser S, Parkinson D (1992). Soil biological criteria as indicators of soil quality: Soil microorganisms. *American Journal of Alternative Agriculture* 7(1-2):33-37. <https://doi.org/10.1017/S0889189300004434>
- Wang C, Liu D, Bai E (2018). Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition. *Soil Biology and Biochemistry* 120:126-133. <https://doi.org/10.1016/j.soilbio.2018.02.003>
- Wang QG, Garrity M, Tiedje JM, Cole JR (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* 73:5261-5267.
- Wang R, Dong LL, Xu J, Chen JW, Li XW, Chen SL (2016). Progress in improvement of continuous monoculture cropping problem in *Panax ginseng* by controlling soil-borne disease management. *China Journal of Chinese Materia Medica* 41(21):3890-3896 (in Chinese with English abstract).
- Wei H, Xu C, Ma L, Duan J, Jiang L, Ren J (2014). Effect of late-season fertilization on nutrient reserves and carbohydrate accumulation in bareroot *Larix olgensis* seedlings. *Journal of Plant Nutrition* 37(2):279-293. <https://doi.org/10.1080/01904167.2013.859697>
- Wei HX, Guo P, Zheng HF, He XY, Wang PJ, Ren ZB, Zhai C (2017). Micro-scale heterogeneity in urban forest soils affects fine root foraging by ornamental seedlings of Buddhist pine and Northeast yew. *Urban Forestry and Urban Greening* 28:63-72. <https://doi.org/10.1016/j.ufug.2017.10.006>
- Xiao C, Yang L, Zhang L, Liu C, Han M (2016). Effects of cultivation ages and modes on microbial diversity in the rhizosphere soil of *Panax ginseng*. *Journal of Ginseng Research* 40(1):28-37. <https://doi.org/10.1016/j.jgr.2015.04.004>
- Xing CH, Cai MZ, Yu HB (2007). Application of EM effective microorganism technology in environmental protection. *Journal of Microbiology* 5:93-97. <http://doi.org/10.3969/j.issn.1005-7021.2007.05.021>
- Zhao J, Li Y, Wang B, Huang X, Yang L, Lan T, Zhang J, Cai Z (2017). Comparative soil microbial communities and activities in adjacent Sanqi ginseng monoculture and maize-Sanqi ginseng systems. *Applied Soil Ecology* 120:89-96. <https://doi.org/10.1016/j.apsoil.2017.08.002>
- Zheng BX, Bi QF, Hao XL, Zhou GW, Yang XR (2017). *Massilia phosphatilytica* sp. nov., a phosphate solubilizing bacteria isolated from a long-term fertilized soil. *International Journal of Systematic and Evolutionary Microbiology* 67(8):2514-2519. <https://doi.org/10.1099/ijsem.0.001916>

Zhu KY, Liu HC, Wei HX, Zhou JH, Zou QC, Ma GY, Zhang JQ (2016). Prediction of nutrient leaching from culture of containerized Buddhist Pine and Japanese Maple seedlings exposed to extended photoperiod. International Journal of Agriculture and Biology 18(2):425-434. <https://doi.org/10.17957/ijab/15.0108>



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