

## Degradation dynamics of nine phenolic acids in American ginseng and maize grown soils

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### ABSTRACT

To get more information about the effects of microorganisms on the phenolic acids degradation, 9-phenolic acids (*p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric, ferulic, benzoic, salicylic, cinnamic acids and vanillin) were added to American ginseng and maize grown soils at two sites (Gejia and Zetou Town, Wendeng District, Weihai City, Shandong Province, China) and determined the residual concentrations of these phenolic acids. All phenolic acids were degraded in 3-days in natural soil and in 9-days in sterilized soil, showing the role of microorganisms in phenolic acid degradation. In addition, crop species had significant effect ( $P < 0.05$ ) on the microbiological degradation rate (MDR) of syringic, *p*-coumaric, salicylic, vanillic acids and vanillin, while soil sampling sites had significant effects ( $P < 0.05$ ) on the MDR of syringic, ferulic and salicylic acids. This study revealed that soil microorganisms mediated by crop species and soil physico-chemical properties affected the degradation of phenolic acids in soil. This finding will aid to understand the interactions between the soil phenolic acids and microorganisms.

**Key Words:** Allelochemicals, American ginseng, maize, MDR, *Panax quinquefolius*, phenolic acids degradation, soil microorganisms, soil physico-chemical properties, soil sterilization, *Zea mays*

### INTRODUCTION

Phenolic acids from the plants or microbial sources are ubiquitous in soil ecosystems and play important roles in soil composition, as these are precursors of soil humic substances (41) and they account for 20 to 30 % of biological carbon cycle in the biosphere (11). Moreover, many *in-vitro* experiments have shown that phenolic acids are important allelochemicals for plants (16,20,26). In the past few years, researchers have been paying more attention to the roles of phenolic acids in soil and found that the allelopathic effects of phenolic acids were decreased in soil due to their degradation (25,43,47). Therefore, understanding the contents and persistence of phenolic acids in soil is very crucial for evaluating their roles in soil.

Phenolic acids are important allelochemicals in soil ecosystems and determining their contents and persistence in soil is crucial to evaluate their role. However, little is known about how preceding crops and soil physico-chemical properties affect the role of soil microorganisms to degrade the phenolic acids in soils. Phenolic acids may be degraded by physical (30,42), chemical (23,40) or biological factors (10,48,49). Many studies have focussed on the degradation of soil phenolic acids. Blum (6) studied the degradation of ferulic acid (FA), demonstrating that soil sorption protects the phenolic

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acids from microbial utilization for short periods of time (6). Liu *et al.* (28) found that benzoic acid (BA) level decreased by 49.18 % of its initial concentration within 48 h in soil. The diverse structures of phenolic acids implicates the possibility of variations in the degradation of individual phenolic acids in soil. However, little is known about the differences in individual phenolic acids degradation with same or different structure of side chains. The soil microorganisms being important determinants of phenolic acids degradation in soil (17), are strongly affected by cultivated plants (2,21) and soil physico-chemical properties (22,35). Therefore, the degradation potential of rhizosphere microbial community of different plant species or the same species cultivated in soil with different physico-chemical properties are variable, but such studies are limited.

In the present study, we used (i) nine-common individual phenolic acids (*p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric, ferulic, benzoic, salicylic, cinnamic acids and vanillin) from different chemical classes as target compounds and (ii) the soils planted with genetically different plants : American ginseng [*Panax quinquefolius*], a perennial dicotyledonous medicinal plant], or maize [*Zea mays*], a monocotyledonous grain crop]. Soil samples with different physico-chemical properties were collected from two sites. This study aimed to find (i). the degradation dynamics of different phenolic acids, (ii). the effects of cultivated plant species and (iii). the effects of soil physico-chemical properties on the microbiological degradation rate, which is very important to understand the interactions between the soil phenolic acids and microorganisms.

## MATERIALS AND METHODS

### Experiment soil

The experimental soil was collected from crop fields of two sites, Liujiatuan village, Zetou town (121° 51' 44" E, 37° 3' 14" N) and Xiaoying village, Gejia town (121° 51' 30" E, 37° 6' 27" N), Wendeng Dist., Weihai City, Shandong Province, China, in July of 2018 during the growth phase of plant. The two sites were cultivated with American ginseng and maize since past 2-years. From each site in 1.0 m<sup>2</sup>, 5-plants each of 2-year-old American ginseng and maize were removed by digging out the whole plant and the soil adhering to roots was collected. These soils were carried to our Institute of Medicinal Plant Development, Beijing. The physico-chemical properties and phenolic acid contents of the experimental soils are summarized in Table 1 and Table 2, respectively.

Half of the soil collected from the field was once sterilized daily by dry-heat method (160°C, 2 h) in hot air 3-consecutive days. Thereafter soil basal respiration was measured to test the effectiveness of sterilization. Soil basal respiration was determined by quantifying the CO<sub>2</sub> released by microbial respiration during 24 h of incubation. Accordingly, 20 g moist soil sample from each pot was incubated in sealed glass jar (250 mL) at 25°C and the amount of CO<sub>2</sub> released was measured after 24 h by alkali absorption (38).

Table 1. The Phenolic acids concentrations in experimental soil

Soil	Cultivated plants	Sampling sites	PHA ( $\mu\text{g g}^{-1}$ )	VA ( $\mu\text{g g}^{-1}$ )	SYA ( $\mu\text{g g}^{-1}$ )	V ( $\mu\text{g g}^{-1}$ )	PA ( $\mu\text{g g}^{-1}$ )	FA ( $\mu\text{g g}^{-1}$ )	SA ( $\mu\text{g g}^{-1}$ )	BA ( $\mu\text{g g}^{-1}$ )	CA ( $\mu\text{g g}^{-1}$ )
G-I	American ginseng	Liyujatuan	1.001	1.438	0.935	0.961	6.789	1.103	2.761	2.632	0.072
G-II	American ginseng	Xiaoying	2.158	3.409	2.600	2.453	18.326	2.056	4.289	5.816	0.115
M-I	Maize	Liyujatuan	1.270	1.936	1.198	1.533	10.898	1.486	3.340	2.910	0.121
M-II	Maize	Xiaoying	0.564	0.827	0.503	0.675	4.997	0.402	1.614	1.040	0.077

G-I: Ginseng cultivated soil of Site I, G-II: Ginseng cultivated soil of Site II, M-I: Maize cultivated soil of Site I, M-II: Maize cultivated soil of Site II.  
 PHA: *p*-Hydroxybenzoic acid, VA: Vanillic acid, SYA: Syringic acid, V: Vanillin, PA: *p*-Coumaric acid, FA: Ferulic acid, SA: Salicylic acid, BA: Benzoic acid, CA: Cinnamic acid

Table 2. The physico-chemical properties of experiment soil

Soil	Total nitrogen ( $\text{g kg}^{-1}$ )	Total phosphorus ( $\text{g kg}^{-1}$ )	Total potassium ( $\text{g kg}^{-1}$ )	Available nitrogen ( $\text{mg kg}^{-1}$ )	Available phosphorus ( $\text{mg kg}^{-1}$ )	Available potassium ( $\text{mg kg}^{-1}$ )	Organic matter (%)	pH	Sand ( $>0.05$ mm) (%)	Silt ( $0.05-0.02$ mm) (%)	Clay ( $<0.02$ mm) (%)
G-I	4.23	1.66	1.59	113.06	83.62	85.89	1.44	5.73	84.87	7.75	7.38
G-II	5.62	1.08	1.50	207.83	29.01	96.41	1.70	5.08	70.04	17.25	12.71
M-I	4.05	1.26	1.42	57.12	51.73	91.35	1.25	5.21	79.21	11.17	9.63
M-II	4.93	0.80	1.22	76.16	17.65	60.51	0.84	5.36	78.37	12.25	9.38

G-I: Ginseng cultivated soil of Site I, G-II: Ginseng cultivated soil of Site II, M-I: Maize cultivated soil of Site I, M-II: Maize cultivated soil of Site II.

### Pot culture

Nine phenolic acids [*p*-hydroxybenzoic acid (PHA), vanillic acid (VA), syringic acid (SYA), vanillin (V), *p*-coumaric acid (PA), ferulic acid (FA), benzoic acid (BA), salicylic acid (SA) and cinnamic acid (CA)], were detected in the American ginseng (14) and maize cultivated soil (Table 1). All nine phenolic acids influenced the ginseng growth (47). The nine phenolic acids used in this experiment were purchased from J&K Scientific (Beijing, China). The phenolic acids were weighed accurately (PHA: 7.2065 g, VA: 4.3204 g, SYA: 4.3247 g, V: 2.8845 g, FA: 1.7900 g, SA: 2.8884 g, BA: 7.2074 g, CA: 0.2861 g, PA: 44.6560g) and dissolved in methanol (1 L for PA and 100 mL for other phenolic acids) to make stock solutions. The stock solutions were kept at 4°C and used within one week. Based on the phenolic acid contents of experimental soil and previously measured soil, the added concentrations of phenolic acids was 40 mg kg<sup>-1</sup> soil (for PHA, VA, SYA and V), 20 mg kg<sup>-1</sup> soil (for FA, BA and SA), 100 mg kg<sup>-1</sup> soil (for PA) and 3 mg kg<sup>-1</sup> soil (for CA). These nine phenolic acid stock solutions were mixed and diluted with sterilized water to obtain mixture of phenolic acids.

Then, 500 mL mixture of phenolic acids was added to 6 kg dry soil per pot with a sprinkling can and mixed thoroughly in the soil, and the water content of soil was adjusted to approximately 20 % (weight/weight). Each of the eight experimental soils (Table 3) were homogenised and divided into four pots (top diameter 15 cm, bottom diameter 10 cm, depth 13 cm). Then, these pots were kept in artificial shade with a double-sunshade cloth in the American ginseng experimental field in our Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College Beijing. The pots were treated on August 28, 2018 and the ambient temperature during the experimental period was 20°C-32°C. After treatment, soil samples (30 g fresh weight) were collected from each pot immediately (day 0) and then after 1, 3, 5, 7 and 9 days. These soil samples were carried to the laboratory and stored at -80°C for extraction of phenolic acids.

Table 3. Treatments of experimental soil

Designation	Sterilization or not	Cultivated plants	Sampling sites
SG-I	Sterilized	American ginseng	Liujiatuan
SG-II	Sterilized	American ginseng	Xiaoying
SM-I	Sterilized	Maize	Liujiatuan
SM-II	Sterilized	Maize	Xiaoying
NG-I	Not sterilized	American ginseng	Liujiatuan
NG-II	Not sterilized	American ginseng	Xiaoying
NM-I	Not sterilized	Maize	Liujiatuan
NM-II	Not sterilized	Maize	Xiaoying

S: Sterilised, N: Not sterilised, G: Ginseng, M: Maize

### Phenolic acids extraction

Before extracting the phenolic acids, each soil sample was divided into two parts: (i) to determine the soil moisture content [dried at 105 °C for 6 h] and (ii) for phenolic acids extraction. Phenolic acids were extracted with modified method of Chen *et al* (10). Six g fresh soil (5 g dry soil equivalent) were added to 30 mL 1 M NaOH and shaken with reciprocal shaker (WR-1, Shanghai S.R.D Scientific Instruments, Shanghai, PR China) for 24 h (170 rpm, 25°C). Then it was centrifuged (5000 g, 15 min) and the supernatant was

adjusted to pH 2.5 with 12 M HCl, stabilized for 2 h at room temperature and centrifuged again to remove any precipitation (5000 g, 15 min). The soil solutions were extracted 5-times with an equal volume of ethyl acetate (28) and dried at 42°C in a rotary vacuum evaporator (EYELA, Tokyo Rikakirai Co., LTD, Japan). The dried residues were re-dissolved in 1.5 mL 80 % methanol and filtered through 0.22 µm nylon membrane for further analysis.

#### **Chromatographic conditions for high-performance liquid chromatography analysis**

Phenolic acids separation was done on HPLC (Agilent1260, Santa Clara, California, USA) equipped with an XDB-C18 (4.6 mm×250 mm, 5 µm) chromatographic column. The UV detection wavelength was set at 254 nm (for PHA, VA and BA) and 280 nm (for SYA, V, PA, FA, SA and CA). The injection conditions and gradient elution system solvent composition were used according to Bi *et al.* (5). Standard stock solutions of 9-phenolic acids were prepared by dissolving each of them in methanol at 50 µg mL<sup>-1</sup> concentration and diluted 2-folds with mixed stock standard solutions (made with methanol). Each concentration of solution was analysed and calibration curves were prepared in the form of regression equation:

$$y = ax + b,$$

Where,  $y$  : Peak area and  $x$  : Concentration of the individual phenolic acids.

#### **Calculation of microbiological degradation rate**

The phenolic acids added to soil decreases, because some are absorbed/adsorbed or degraded by abiotic factors (30,42) and some are metabolized by microorganisms (19). We considered the portion metabolized by microorganisms as microbiological degradation. Therefore, the microbiological degradation rate (MDR) can be defined as, the reduced concentration of phenolic acids in the soil per unit time due to microbial metabolism. The MDR was calculated as under:

$$\text{MDR} = \frac{RCS(x) - RCN(x)}{x} \times 100\%$$

Where, MDR : Microbiological degradation rate of phenolic acids,  $x$  : Days,  $RCS(x)$  : Residual concentrations of phenolic acids in sterilized soil,  $RCN(x)$  : Residual concentrations of phenolic acids in natural soil after  $x$  days.

#### **Bacterial and fungal community analysis**

The bacterial and fungal communities were analysed following procedure of DNA extraction, PCR amplification and Illumina MiSeq sequencing. DNA was extracted from 0.4 g soil sample using the MOBIO PowerSoil® DNA Isolation Kit (Mobio Laboratories Inc., Carlsbad, CA, USA). The bacterial universal V3-V4 region of the 16S rRNA gene was amplified with amplicon PCR forward primer (5'-ACTCCTACGGGAGG CAGCAG-3') and amplicon PCR reverse primer (5'-GGACTACHVGGGTWTCTAAT-3') (50). The fungal universal ITS1 region was amplified with the amplicon PCR forward primer (5'-CTTGGTCATTTAGAGGAAGTAA-3') and amplicon PCR reverse primer (5'-TGCGTTCCTCATCGATGC-3') (34). Three PCR products per sample were pooled, purified and quantified by real-time PCR. Parallel-tagged sequencing was done on Illumina MiSeq platform (Allwegene, Beijing, China) according to standard protocols (13,46). Specifically, split reads were merged using FLASH V1.2.11 and sorted into each sample by the unique barcodes using QIIME (V1.7.0). The raw data was first screened and sequences were removed by considering, whether their quality scores < 20 contained

ambiguous bases or did not exactly match the primer sequences and barcode tags. Raw tags with < 200 bp were removed using Mothur (V1.37.0). Chimeras were removed with USEARCH (V8.1.1861) against the Gold and UNITE reference database. The sequences were clustered into operational taxonomic units (OTUs) at threshold of 97 % similarity using the UPARSE pipeline. Singletons OTUs were removed from the subsequent analyses to reduce over prediction of rare OTUs. The representative OTU sequences were aligned and annotated using the Ribosomal Database Project (RDP) classifier (V14). The coverage, Chao1, phylogenetic (PD) and Shannon indices were analysed using QIIME (V1.8) (39). The heatmap was plotted with R package pheatmap.

### Statistical analysis

The concentrations of phenolic acids in fresh soil were converted into  $\mu\text{g g}^{-1}$  dried soil. SPSS software (version 19.0, SPSS Inc., Chicago, IL, USA) was used for data analysis. The means and standard deviations (SD) of phenolic acid contents were calculated. One-sample *t*-tests (two-tailed) were conducted to determine whether the basal respiration of sterilized soil differed from zero (the test value set at 0 and the significance level set at  $P < 0.05$ ). The MDR of phenolic acids was subjected to analysis of variance and compared by Duncan's multiple range tests. The double factor analysis was confirmed by a general linear model.

## RESULTS AND DISCUSSION

### Test of the sterilization effect

The sterilization effect was tested by measuring the basal respiration of soil. The significant values of one-sample *t*-tests for SG-I (sterilized ginseng cultivated soil collected at Site I), SM-I (sterilized maize cultivated soil collected at Site I), SG-II (sterilized ginseng cultivated soil collected at Site II) and SM-II (sterilized maize cultivated soil collected at Site II) were 0.581, 0.424, 0.345 and 1.000, respectively, indicating that there was no significant difference ( $P > 0.05$ ) between the zero and the mean value of basal respiration for each sterilized soil. Therefore, the sterilization effect was acceptable for the following experiment.

### Phenolic acid degradation dynamics in sterilized or unsterilized soil

Nine individual phenolic acids, PHA, VA, SYA, PA, FA, BA, SA, CA and V were added to sterilized or natural soil. The degradation curves of the 9-individual phenolic acids are shown in Figure 1a (for soil of Site I) and Figure 1b (for soil of Site II). In general, the degradation curves were similar between the two sites. It can be seen from Figure 1a and 1b that the degradation curves become steady, when the phenolic acids were degraded to the turning point (TP) and all nine individual phenolic acids could be degraded to the TP in the eight groups within 9-days. The TPs for PHA, VA, SYA, V, FA, SA and BA were in the range of 0-5  $\mu\text{g g}^{-1}$ ; for PA, the TP was 5-20  $\mu\text{g g}^{-1}$  while for CA, it was 0-0.25  $\mu\text{g g}^{-1}$  (Figure 1), which were close to the background values of soil (Table 1). It can be inferred that the phenolic acids cannot be fully degraded. This phenomenon was explained by Dalton (12), who purposed that sorption of phenolic acids by soils may provide some protection to phenolic acids from degradation.

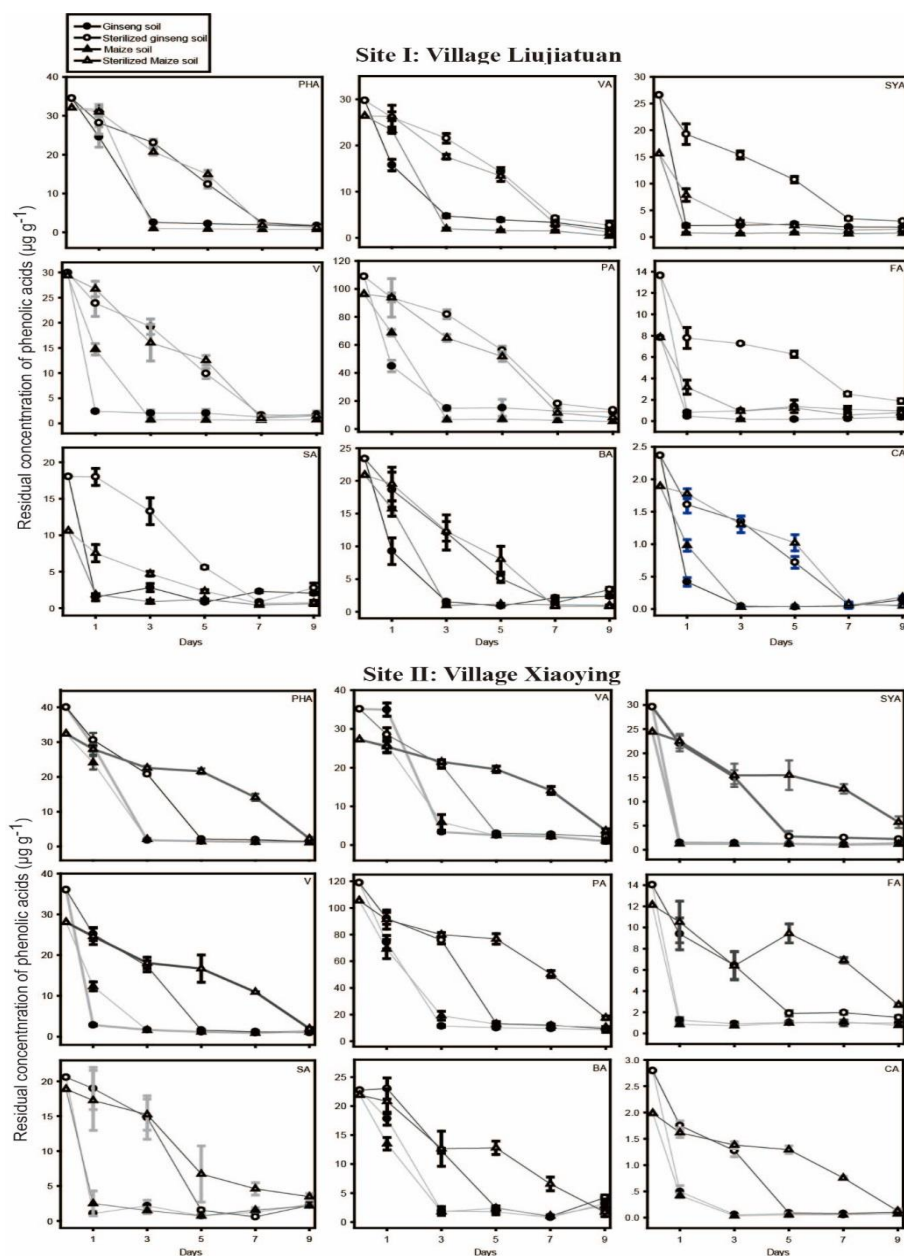
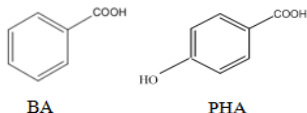
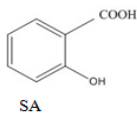
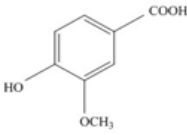
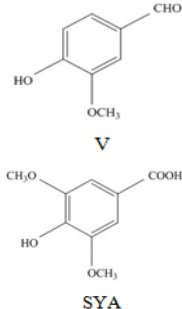
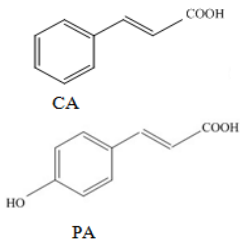
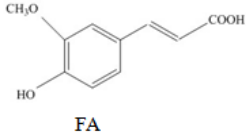


Figure 1. Degradation dynamics of the nine phenolic acids in soil collected at Site I and Site II. Bars indicate the standard deviation (SD) of four replicates. The absence of error bars indicates that the error bars are smaller than the symbol representing the mean. PHA: *p*-Hydroxybenzoic acid, VA: Vanillic acid, SYA: Syringic acid, V: Vanillin, PA: *p*-Coumaric acid, FA: Ferulic acid, SA: Salicylic acid, BA: Benzoic acid, CA: Cinnamic acid.

Table 4. Chemical structures of test phenolic acids after 1- and 3-days of degradation to turning point of degradation curve in natural soil

Class of phenolic acids	Degradation days to turning point of degradation curve	
	3-days	1-day
Benzoic acid derivatives without methoxy group	 <p>BA                      PHA</p>	 <p>SA</p>
Benzoic acid derivatives with methoxy group	 <p>VA</p>	 <p>V SYA</p>
Cinnamic acid derivatives	 <p>CA                      PA</p>	 <p>FA</p>

After turning point, the degradation rates become low and the degradation curves become steady.

In natural soil collected from both sites, the degradation curves of PHA, VA, PA, BA and CA degraded to their TPs on the third day, while this occurred in only one day for SYA, V, FA and SA (Figure 1a and 1b). Our results regarding BA were consistent with the study of Liu *et al.* (28), where BA was degraded upto 20 % within 3-days of addition in soil. However, the degradation of FA in our experiment showed variation from the previous studies, where the residual concentration of FA was approximately half of the initial concentration after the soil was infected with microbes for 40 h (6). The deviation in results might be attributed to the fact that Blum (6) inoculated microbes into sterilized soil by adding soil extracts, while in present study natural soil was used to evaluate the degradation of phenolic acids. According to the structures of the 9- phenolic acids, they can be classified into three categories: (i). Benzoic acid derivatives-without methoxy groups, (ii). Benzoic acid derivatives with-methoxy groups and (iii). Cinnamic acid

derivatives (Table 4). For SA, the hydroxyl group in the ortho-position of the carboxyl group makes this phenolic acid more active than PHA and BA. For FA, the methoxy group may make this phenolic acid more easily degraded than other two cinnamic acid derivatives, because the methoxy group is more vulnerable to degradation than the hydroxy group in soil (18). With respect to SYA, its additional methoxy group might be responsible for its faster degradation than VA (Table 4). In addition, we speculated that V can be oxidized to VA in soil, which made it disappear faster than V; however, more evidences are still needed to prove that.

Figure 1 also shows that the phenolic acids in unsterilized soil degraded faster than those in sterilized soil, regardless of the sites and crops cultivated. The degradation curves of unsterilized soil stabilized within 3-days, but for sterilized soil, this took 5-9 days (Figure 1), which implies that soil microorganisms play very important role in phenolic acid degradation. Li *et al.* (24) studied the degradation dynamics of 6-individual phenolic acids in solution and found that phenolic acids decreased by 70 % after seven days, which is similar to the sterilized soil in our study, indicating that few microorganisms degrade the phenolic acids in solution. Bi *et al.* (4) studied the degradation of total phenolics in soil and found that phenolics degraded faster in natural soil than in sterilized soil. In this study, it was observed that nine phenolic acids were quickly metabolized by soil microorganisms. Even without microorganisms, phenolic acids were also degraded. The chemicals in soil, such as iron and manganese oxides might be responsible for gradual oxidation of phenolic acids (23,31). This evidence explained the decrease in phenolic acids in sterilized soil in our experiments (Figure 1). Our results indicated that the quick degradation of phenolic acids in soil reduces their allelopathic effects on plants.

#### MDR of phenolic acids and its influence factors in soil

In unsterilized soil, five individual phenolic acids (i.e., BA, PHA, VA, CA and PA) reach TP in 3-days, while four individual phenolic acids (SA, V, SYA and FA) reach TP in 1-day (Figure 1 and Table 4). Therefore, we calculated the MDR within three days for five individual phenolic acids and within one day for four individual phenolic acids. The MDR of the 9-individual phenolic acids are summarized in Table 5. MDR reflects the capacity of microorganisms to utilize phenolic acids, excluding the physical and chemical factors.

Table 5. Microbiological degradation rate of 9- individual phenolic acids in different soils

Designation	Microbiological degradation rate ( $\mu\text{g g}^{-1} \text{d}^{-1}$ )								
	PHA	VA	SYA	V	PA	FA	SA	BA	CA
G-I	6.3±0.10b	5.8±0.25a	20.5±1.59a	22.3±1.64a	21.5±0.80ab	8.1±1.27ab	17.9±2.72a	3.5±0.29a	0.41±0.04a
G-II	6.8±0.30a	5.6±0.35ab	17.1±1.88b	21.5±2.80a	22.3±1.44a	7.0±1.00b	16.5±0.83a	3.5±0.92a	0.44±0.03a
M-I	6.8±0.13a	5.2±0.50b	21.3±1.43a	12.3±3.05b	20.3±0.88bc	9.7±1.89a	14.8±3.96a	3.6±0.90a	0.45±0.02a
M-II	6.6±0.30ab	5.2±0.17b	7.1±1.21c	12.0±1.55b	19.5±0.99c	2.7±0.68c	5.7±1.26b	3.8±0.49a	0.42±0.04a
Significance due to									
Crop Species	NS	*	*	*	*	NS	*	NS	NS
Sampling Sites	NS	NS	*	NS	NS	*	*	NS	NS
CS×SS	*	NS	*	NS	NS	*	*	NS	NS

Data are the means  $\pm$  SD (n=4). Values in the same column marked with the same letter are not significantly different ( $P > 0.05$ , compared by Duncan's multiple range tests). NS stands for not significant; \* stands for  $P < 0.05$ , confirmed by general linear model, CS×SS means interaction between s crop species and sampling sites, G-I: Ginseng cultivated soil of Site I, G-II: Ginseng cultivated soil of Site II, M-I: Maize cultivated soil of Site I, M-II: Maize cultivated soil of Site II.

### **(i) Influences of-crop species on MDR**

The double-factor analysis showed that the crop species had no significant effects on the MDRs of FA, PHA, BA and CA ( $P > 0.05$ ). However, for SA, PA, VA and V the preceding crop species and soil had significant effects on MDRs. The MDRs of American ginseng-cultivated soil were higher than those of maize-cultivated soil sampled at the same site. For SYA, the crop species also had significant effects on the MDR, and the MDR of American ginseng-cultivated soil was significantly higher ( $P < 0.05$ ) than maize-cultivated soil from Site II, while, there were no significant differences between the two crops-cultivated soil collected from Site I (Table 5).

In previous studies, Liste and Alexander (27) have reported that plants can promote pyrene degradation in soil and the degradation rates among different species were variable, which is very similar to our results about the degradation of phenolic acids. Mala *et al.* (32) investigated the influence of woody plant species on phenolic acid contents in soil and reported that the concentrations of phenolic acids varied in soil under different trees. Liu *et al.* (29) also found that the phenolic acids differed in rhizosphere of various species of mangrove plants and speculated that the differences were due to synthetic effects of different secretions of plants, utilization by microorganisms and adsorption in soil. In our study, after excluding the plants secretion and soil adsorption, we can conclude that the rates of utilization of phenolic acids by microorganisms harbouring various plants species in cultivated soils are different. For FA, PHA, BA and CA, crop species had no significant effect on the MDR, indicating that the functions of the microorganisms that metabolise these phenolic acids may be similar. It can be inferred that microorganisms in American ginseng-cultivated soil are more capable of metabolizing SA, PA, VA and V compared to maize-cultivated soil.

### **(ii) Effects of soil physico-chemical properties on MDR**

In addition to crop species, our results also revealed that sampling sites affected the MDR. According to double-factor analysis, the sampling sites of soil had significant effect on the MDRs of SYA, FA and SA ( $P < 0.05$ , Table 5). The MDRs of SYA and FA were higher in soil of Site I than Site II. Moreover, the sampling sites and crop species had significant interaction effects on the MDRs of PHA, SYA, FA and SA ( $P < 0.05$ ). Obviously, the soil collected from various sites had different physico-chemical properties (Table 2). Accordingly, we can speculate physico-chemical properties affects the MDR of phenolic acids.

The biodegradation of phenolics is affected by soil physico-chemical properties (6,7), which were consistent with our findings. In fact, the process of soil phenolic acid metabolism is dependent on soil microorganisms (1), which are affected by soil physico-chemical properties. Besides, plants, rhizosphere microorganisms and soil physico-chemical properties interact with each other (3).

### **(iii) Influences of crop species and soil physico-chemical properties on MDR**

Across all the samples, when using the 97 % sequence similarity cutoff, 51032-98168 clean tags of 16S were obtained and grouped into 1091-1500 bacterial OTUs; 64661-92248 clean tags of internal transcribed spacer (ITS) were obtained and grouped into 492-526 fungal OTUs. The diversity indices of soil bacteria and fungi were summarized in Table 6. The coverage indices for bacteria and fungi were between a range of 0.9951-0.9959 and 0.9980-0.9985, respectively (Table 6), suggesting that the

sequencing capability was large enough to capture the majority of the diversity for all samples. For bacteria, the Chao1, phylogenetic indices and Shannon of G-I, M-I and G-II were higher than that of M-II, indicating that the bacterial richness, phylogenetic diversity and evenness in M-II were lower than other three groups. For fungi, the Chao1 and phylogenetic indices of G-I and G-II were higher than that of M-I and M-II, indicating that the richness and phylogenetic diversity of American ginseng cultivated soil were higher than maize cultivated soil, while the Shannon of G-II and M-II were higher than G-I and M-I. According to average abundance, the top 18 genera of bacteria and fungi are shown in Figure 2.

Table 6. Bacterial and fungal diversity of experimental soil

Designation	Bacteria				Fungi			
	Coverage	Chao1	PD	Shannon	Coverage	Chao1	PD	Shannon
G-I	0.9959	1582	104	8.6	0.9980	598	101	5.0
G-II	0.9951	1469	92	8.1	0.9985	595	101	5.7
M-I	0.9954	1514	99	8.5	0.9981	562	92	5.0
M-II	0.9954	1225	79	7.4	0.9983	575	94	5.6

G-I: Ginseng cultivated soil of Site I, G-II: Ginseng cultivated soil of Site II, M-I: Maize cultivated soil of Site I, M-II: Maize cultivated soil of Site II.

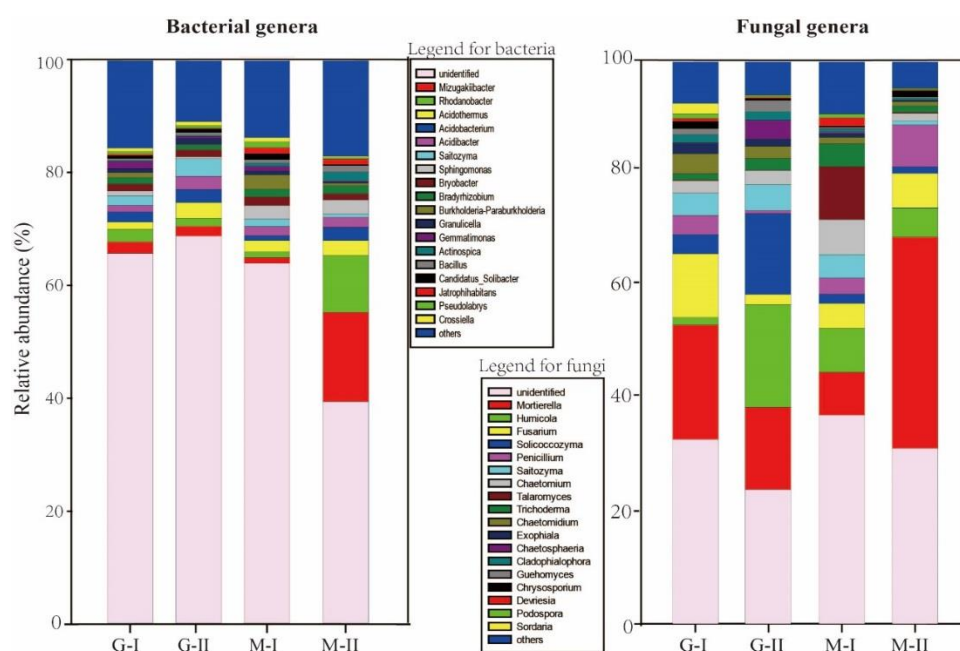


Figure 2. Relative abundance (%) of the major bacterial and fungal genera of soil.

**Bacteria:** For bacteria, the identified composition account for 31-61 % of all the taxa. *Mizugakiibacter* and *Rhodanobacter* were dominant in M-II and the relative abundance was 5 to 12 times higher than that of the other three groups. The relative abundance of *Rhizomicrobium*, *Granulicella*, *Candidatus*, *Solibacter*, *Pseudolabrys* and *Crossiella* in M-II was much lower than other three groups. For the rest of bacterial genera, the relative abundances were even or similar in general.

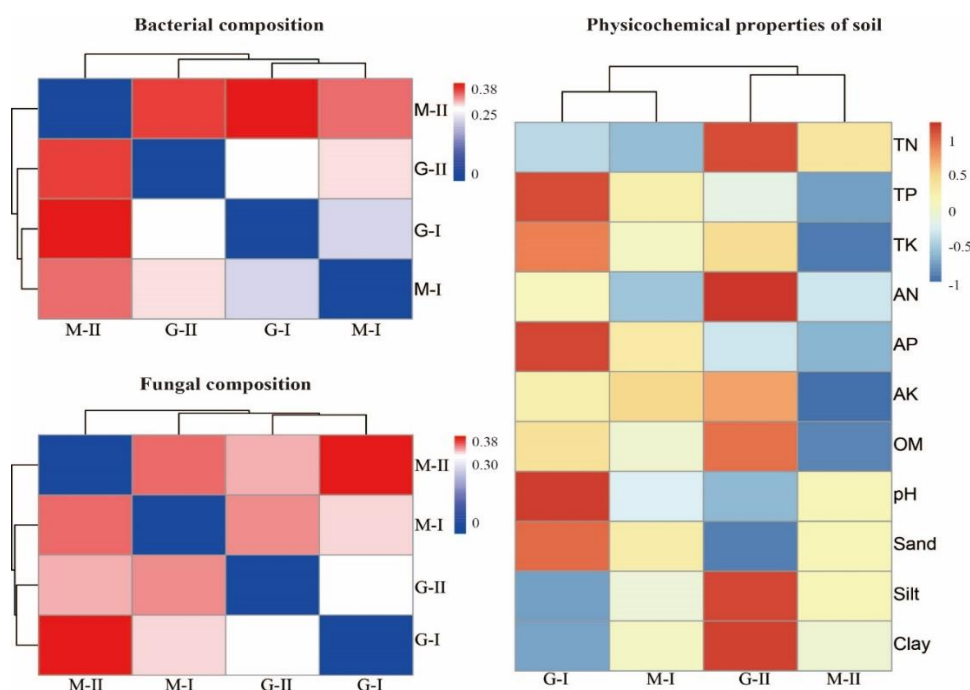


Figure 3. Changes in microbial community and physicochemical properties of different soil samples. Clustering on the axis is based on unweighted Unifrac distance of microbial composition at genera level and similarity of physicochemical properties of soil samples. G-I: Ginseng cultivated soil of Site I, G-II: Ginseng cultivated soil of Site II, M-I: Maize cultivated soil of Site I, M-II: Maize cultivated soil of Site II, TN: total nitrogen, TP: total phosphorus, TK: total potassium, AN: available nitrogen, AP: available phosphorus, AK: available potassium, OM: organic matter.

**Fungi:** With respect to fungi, the identified composition account for 63-69 % of all the taxa. The different taxa varied highly in different groups. *Mortierella* dominated in M-II (relative abundance: 38 %) and G-I (relative abundance: 20 %), *Humicola*, *Mortierella* and *Solicoccozyma* dominated in G-II (relative abundance: 14-18 %), while *Humicola*, *Mortierella*, *Chaetomium* and *Talaromyces* dominated in M-I (relative abundance: 6-9 %). Besides, M-II, G-II and M-I contained the highest relative abundance of *Fusarium*, *Penicillium* and *Trichoderma*, respectively.

The heat map based on unweighted Unifrac distance showed that the general distance of M-II was far from the other three groups for both bacterial and fungal composition. The G-I and M-I clustered together firstly for bacteria, while G-I and G-II clustered together firstly for fungal composition (Fig. 3a, 3b).

The collective genome of the plant rhizosphere microbiota is referred to as the plants' second genome (2), which can be shaped by plant root exudates (15,37). The rhizosphere microorganisms vary in different plants (44). Being perennial plants, American ginseng can influence its rhizosphere microorganisms for longer period than maize, so the fungal composition of American ginseng cultivated soil collected at the two sites were similar (Figure 3b) and their fungal Chao1 and phylogenetic indices were higher than that of maize cultivated soil (Table 6). Moreover, soil provides food source and residence to the microorganisms, so the soil physico-chemical properties could strongly influence its microorganisms (22,35). Although the soil physico-chemical properties of the four groups were different, the clustering on heat map showed that the physico-chemical properties of soil collected from the same site was similar and the physico-chemical properties of soil collected from Site I was more similar than Site II (Figure 3c). Being affected by the soil physico-chemical properties, the bacterial composition of two plants cultivated soil collected at Site I was similar (Figure 3a) and the Chao 1, phylogenetic and Shannon indices of soil at Site I were higher than that of soil at Site II. In fact, both bacteria and fungi are affected by cultivated plants and soil physico-chemical properties, although their sensitivity differs to various factors.

Previous studies have elaborated the relationship between the microbial diversity and its functions (33). Cao *et al.* (8,9) demonstrated that higher diversity of microorganisms could increase the degradation of oxytetracycline. The above studies were consistent with our results and partly support our findings that the diversity of microorganisms influenced their capabilities to degrade phenolic acids, but more soil samples were needed to generalize the findings. Since many genera could not be identified with the 16S rRNA or ITS gene sequencing, we did not find many microorganisms degrading the phenolic acids as previously reported, especially the microorganisms of relative high abundance (*Mizugakiibacter*, *Rhodanobacter*, *Mortierella* and *Humicola*). Only *Bacillus* (36), *Fusarium* (45) and *Trichoderma* (10) metabolized the phenolic acids in our study. Notwithstanding its limitations, our study provided method to study the influence of cultivated plants and soil physico-chemical properties on the microbiological degradation process of phenolic acids.

## CONCLUSIONS

Our results demonstrated that microbiological degradation accounts for large part of phenolic acid degradation, confirming the important role of microorganism during the phenolic acids degradation in soil. Moreover, both plant species (American ginseng and maize) and soil physico-chemical properties had strong influence on the microbiological degradation rate of phenolic acids by mediating the soil microbial communities. These findings will aid to understand the interactions between the soil phenolic acids and microorganisms.

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