Syringic acid inhibited cucumber seedling growth and changed rhizosphere microbial communities

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ABSTRACT

Phenolic compounds enter soil as a result of root exudation and plant residue decomposition, but their impacts on soil microbial communities are poorly understood. In this experiment, effects of syringic acid on cucumber rhizosphere microbial communities were evaluated. Rhizosphere bacterial and fungal community structures and abundances were analyzed with PCR-denaturing gradient gel electrophoresis (DGGE) and quantitative PCR, respectively. Results showed that syringic acid inhibited cucumber seedling growth at concentrations of 0.05 to 0.2 μmol/g soil, and increased rhizosphere soil dehydrogenase activity, microbial biomass carbon content, bacterial 16S rRNA gene and fungal ITS rRNA gene densities, and decreased the bacteria-to-fungi ratio at concentrations of 0.02 to 0.2 μmol/g soil. Syringic acid also changed rhizosphere bacterial and fungal community structures: it decreased the richness, evenness, and diversity indices of rhizosphere bacterial community but had no significant influences on that of fungal community, indicating that syringic acid had different influence on bacterial and fungal communities. Taken together, these results showed that syringic acid inhibited cucumber growth and altered rhizosphere microbial communities, suggesting that syringic acid plays some role in the communication between plants and soil microorganisms.

Keywords: allelopathy; *Cucumis sativus* L.; microbial community abundance and structure; phenolic compound

Phenolic compounds, an important class of plant secondary metabolites, can enter the soils as a result of root exudation and plant residues decomposition (Bais et al. 2006). Various phenolic compounds were identified in the root exudates or decomposing plant debris of several plant species (Yu and Matsui 1994, Zhou et al. 2012). In some cases, phenolic compounds were implicated as allelochemicals in the rhizosphere and have multifunctional roles in below-ground plant-microorganism interactions (Bais et al. 2006, Inderjit et al. 2009).

Through releasing allelochemicals, a plant species can inhibit the growth of plants of the same species, a kind of intraspecific allelopathy which was termed as 'autotoxicity' (Singh et al. 1999). Phenolic compounds are usually mentioned as the causing agents of autotoxicity in several plants (Yu and Matsui 1994, Zhou et al. 2012). After they enter the soil, phenolic compounds would meet numerous and diverse soil microorganisms. On one hand, soil microorganisms can transform or utilize phenolic compounds, and thus influence

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the persistence, availability and biological activities of these compounds. On the other hand, phenolic compounds can affect soil microorganisms (Inderjit et al. 2009). Hence, it is argued that soil microorganisms can act as the targets and mediators of allelopathy in plants (Cipollini et al. 2012). Effects of phenolic compounds on the growth of specific microorganisms, such as *Pseudomonas syringae* and *Fusarium oxysporum*, were investigated *in vitro* (Bais et al. 2005, Lanoue et al. 2010). However, how these compounds affect soil microbial communities *in vivo* was largely unstudied.

Cucumber is a crop of high economic importance in many countries. Phenolic compounds can accumulate in the soil after continuous cropping of cucumber and have detrimental effects on cucumber growth (Yu and Matsui 1994, Zhou et al. 2012). Syringic acid (SA) was identified in the cucumber-cultivated peat-bark substrate (Politycka et al. 1984) and soils under cucumber (Zhou et al. 2012). Here, we focused on the effects of SA on whole rhizosphere microbial communities. Cucumber seedlings were treated with SA (0.02, 0.05, 0.1, 0.2 μmol/g soil) every other day. Rhizosphere microbial communities were analyzed ten days after the treatment.

MATERIAL AND METHODS

Greenhouse experiment. The soil used was collected from the upper soil layer (0–15 cm) of an open field in the experimental station of the Northeast Agricultural University, Harbin, China (45°41'N, 126°37'E), which was covered with grass and undisturbed for more than 15 years. The soil had sandy loam texture, contained organic C, 21.2 g/kg; NH_4^+-N and NO_3^--N, 89.02 mg/kg; available P, 63.36 mg/kg; available K, 119.15 mg/kg; electrical conductivity (EC, 1:2.5, w/v), 0.33 mS/cm; and pH (1:2.5, w/v), 7.78. Cucumber seedlings (cv. Jinlv 3) with two cotyledons were transplanted into cups contained 150 g soil. Fertilizer was not added. There was one seedling per cup. These seedlings were maintained in a greenhouse (32°C day/22°C night, relative humidity of 60–80%, 16 h light/8 h dark).

Cucumber seedlings at the one-leaf stage were treated with different concentrations of SA (0.02, 0.05, 0.1, 0.2 μmol/g soil) every two days (a total of five treatments) as described by Blum et al. (1987). The final concentration added in each treatment was 0.1, 0.25, 0.5, 1.0 μmol/g soil, respectively. The solution pH was adjusted to 7.0 with 0.1 mol/L NaOH solution, because the soil pH is widely accepted as a dominant factor that regulates soil microbial communities (Fierer and Jackson 2006). The soil treated with distilled water was used as the control. Soil water content was adjusted every two days with distilled water to maintain a constant weight of cups. Each treatment had five plants and was done in triplicate.

Cucumber seedling dry weight measurement. Ten days after the first application of SA, the whole cucumber plant was harvested. Plant dry weight was measured after oven drying at 70°C to constant weight.

Rhizosphere soil sampling. Cucumber rhizosphere soils were collected from five plants in each replicate as described before (Zhou and Wu 2012). Part of these fresh soils was used for soil dehydrogenase activity and microbial biomass carbon (MBC) content estimation, and the other part was stored at –70°C for DNA extraction.

Rhizosphere soil dehydrogenase activity and MBC content estimation. Dehydrogenase activity was determined by the reduction of 2,3,5-triphenyltetrazolium chloride method (Tabatabai 1994). MBC content was determined by the chloroform-fumigation-extraction method, and an extractability factor of 0.38 was used to calculate MBC (Vance et al. 1987).

DNA extraction and PCR-denaturing gradient gel electrophoresis (DGGE). Rhizosphere bacterial and fungal community structures were analyzed with the PCR-DGGE method. Total soil DNA was extracted with the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, USA). PCR amplification of the partial bacterial 16S rRNA gene was performed with the primer set of GC-338f/518r (Muyzer et al. 1993). A nested PCR protocol was used to amplify the fungal internal transcribed spacer (ITS) regions of the rRNA gene with primer sets of ITS1F/ITS4 and GC-ITS1F/ITS2 for the first and second round of PCR amplifications, respectively (White et al. 1990, Gardes and Bruns 1993). PCR and DGGE were performed according to Zhou et al. (2012).

Quantitative PCR assay. Abundances of rhizosphere bacterial and fungal communities were estimate by quantitative PCR assays with primer sets of 338f/518r (Muyzer et al. 1993) and ITS1F/ITS4 (White et al. 1990, Gardes and Bruns 1993) as described before (Zhou and Wu 2012, Zhou et al. 2014).

Statistical analysis. Data were analyzed following analysis of variance (ANOVA) and mean comparison between treatments was performed based on the Tukey's honestly significant difference (*HSD*) test at the 0.05 probability level with SAS software (version 8.0, SAS institute, Cary, USA). Banding patterns of the DGGE profiles and principal component analysis (PCA) were analyzed by the Quantity One software (version 4.5, Bio-Rad Laboratories, Hercules, USA) and Canoco for Windows 4.5 software (Plant Research International, Wageningen, the Netherlands), respectively. The richness (S), evenness (E), and diversity (H) indices were calculated as described before (Zhou et al. 2012).

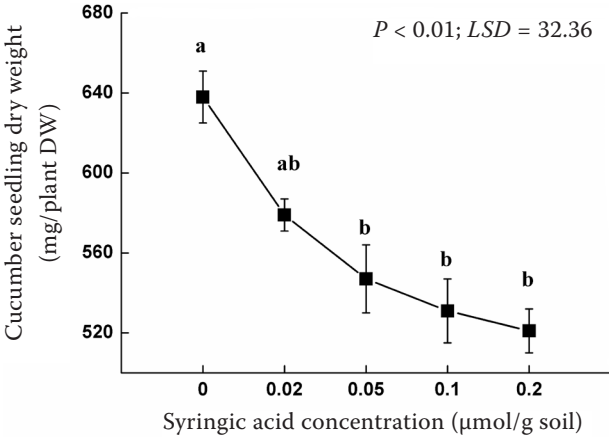

Figure 1. Effects of syringic acid on cucumber seedling dry weight (DW). Values (mean ± SE) with different letters are significantly different at the 0.05 probability level (Tukey's *HSD* test). *P* is from one-way ANOVA

RESULTS

Cucumber seedling dry weight. Cucumber seedling dry weight was significantly influenced by SA ($P < 0.01$) (Figure 1). SA significantly inhibited cucumber seedling dry weight at concentrations of 0.05, 0.1 and 0.2 μmol/g soil ($P < 0.05$). Compared with the control,

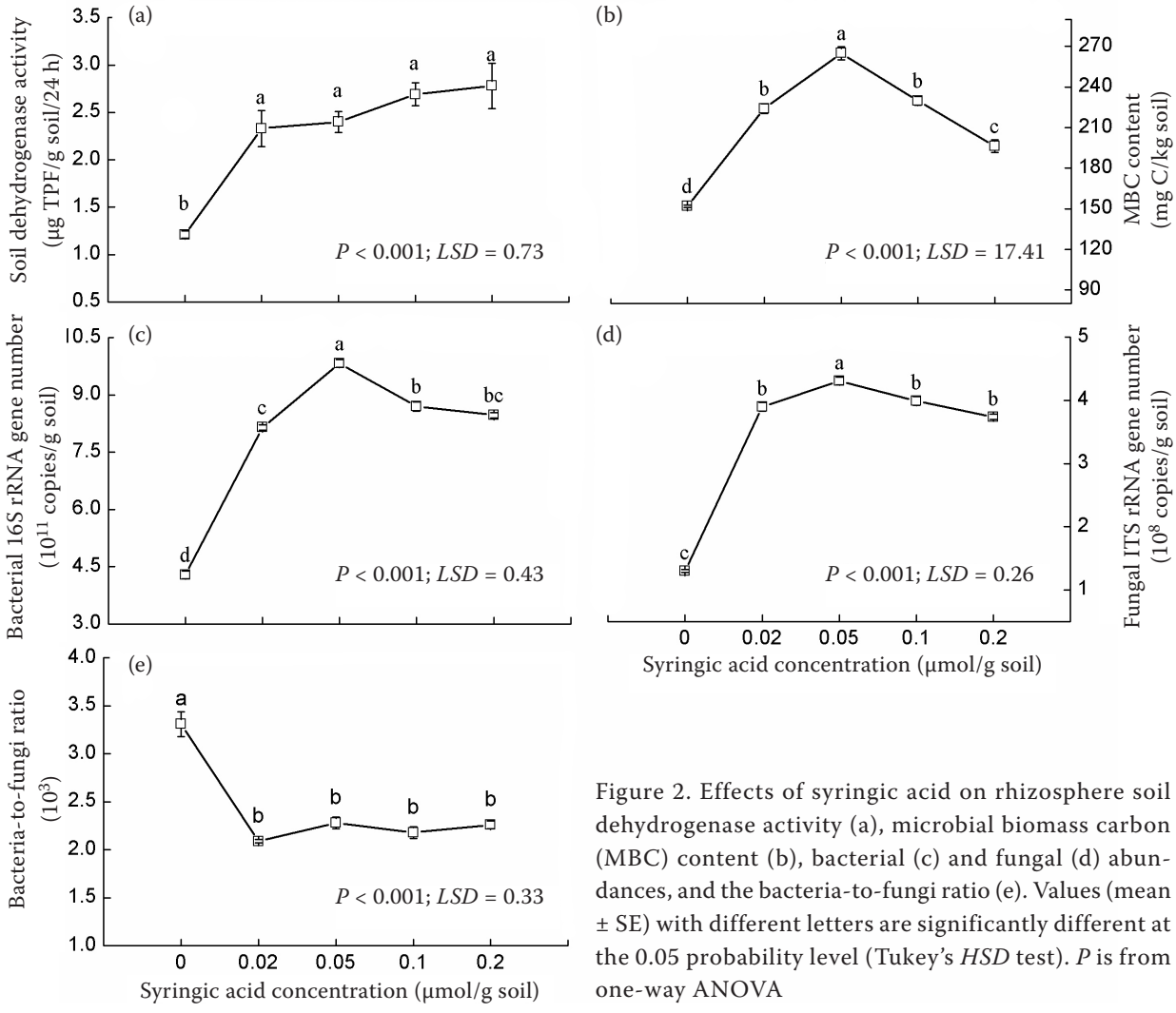

Figure 2. Effects of syringic acid on rhizosphere soil dehydrogenase activity (a), microbial biomass carbon (MBC) content (b), bacterial (c) and fungal (d) abundances, and the bacteria-to-fungi ratio (e). Values (mean ± SE) with different letters are significantly different at the 0.05 probability level (Tukey's *HSD* test). *P* is from one-way ANOVA

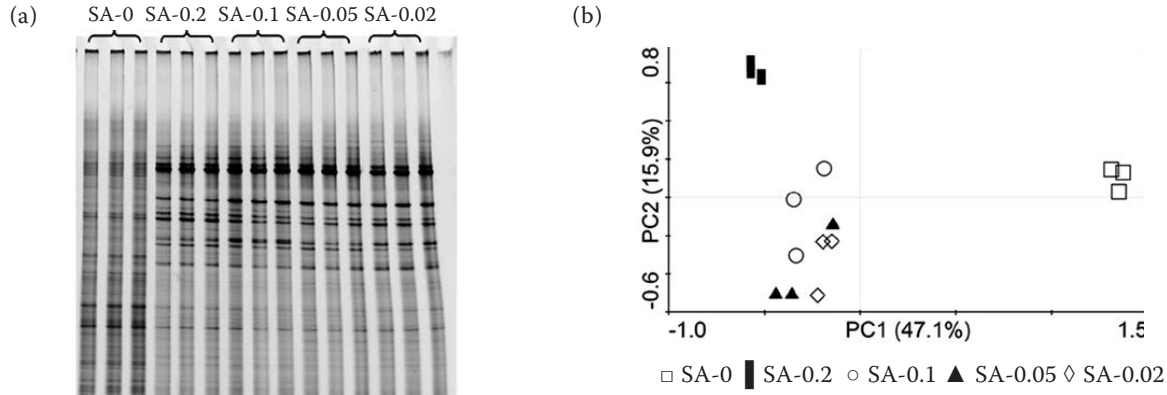

Figure 3. DGGE profile (a) and PCA analysis (b) of bacterial community. Syringic acid (SA)-0, SA-0.02, SA-0.05, SA-0.1 and SA-0.2 represent soil treated with SA at the concentration of 0, 0.02, 0.05, 0.1, 0.2 μmol/g soil, respectively

cucumber seedling dry weight had a reduction of 18.3% at the concentration of 0.2 μmol/g soil.

Rhizosphere soil dehydrogenase activity and MBC content. SA significantly increased dehydrogenase activity (Figure 2a) and MBC content (Figure 2b) in the rhizosphere ($P < 0.001$ and $P < 0.001$, respectively). Significant stimulation of dehydrogenase activity was found even at the lowest concentration (0.02 μmol/g soil), which was about 0.92 times more than the control. MBC content was the highest at 0.05 μmol/g soil, which was about 0.74 times more than the control.

Rhizosphere bacterial and fungal community abundances. SA significantly increased rhizosphere bacterial (Figure 2c) and fungal (Figure 2d) community abundances ($P < 0.001$ and $P < 0.001$, respectively) and decreased bacteria-to-fungi ratio ($P < 0.001$) (Figure 2e). SA at 0.05 μmol/g soil had the largest bacterial and fungal community abundances among all treatments.

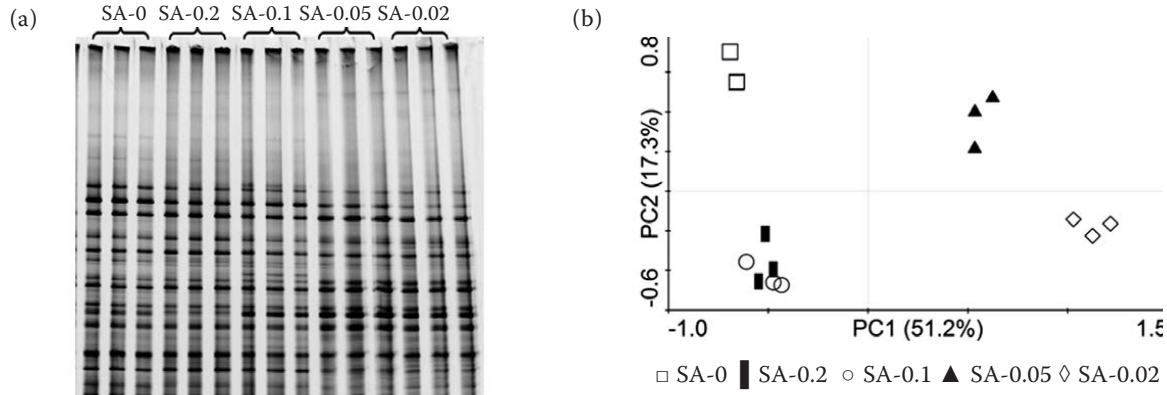

Figure 4. DGGE profile (a) and PCA analysis (b) of partial fungal community. Syringic acid (SA)-0, SA-0.02, SA-0.05, SA-0.1 and SA-0.2 represent soil treated with SA at the concentration of 0, 0.02, 0.05, 0.1, 0.2 μmol/g soil, respectively

Rhizosphere bacterial and fungal community structures. PCR-DGGE analyses showed that SA obviously changed rhizosphere bacterial (Figure 3a) and fungal (Figure 4a) community structures. Visual inspection of these profiles found that DGGE banding patterns were broadly similar in triplicate samples of each treatment for both bacterial (Figure 3a) and fungal (Figure 4a) communities, while DGGE banding patterns were different between the control soil and the soils treated with SA. PCA analyses of rhizosphere bacterial (Figure 3b) and fungal (Figure 4b) DGGE profiles clearly separated SA-treatments from the control.

SA significantly decreased the number of visible bands, Shannon-Wiener index and evenness index of the bacterial community structure ($P < 0.01$, $P < 0.001$ and $P < 0.001$, respectively) (Figure 5a, c, e). However, for fungal community structure, SA showed no significant effects on the number of visible bands and Shannon-Wiener index (Figure 5b, d), but inhibited the evenness index only at 0.02 μmol/g soil ($P < 0.05$) (Figure 5f).

DISCUSSION

Previous studies found that phenolic compounds rapidly disappeared from soil solution as a result of retention by soil particles, utilization by microbes and/or uptake by plant roots (Blum et al.

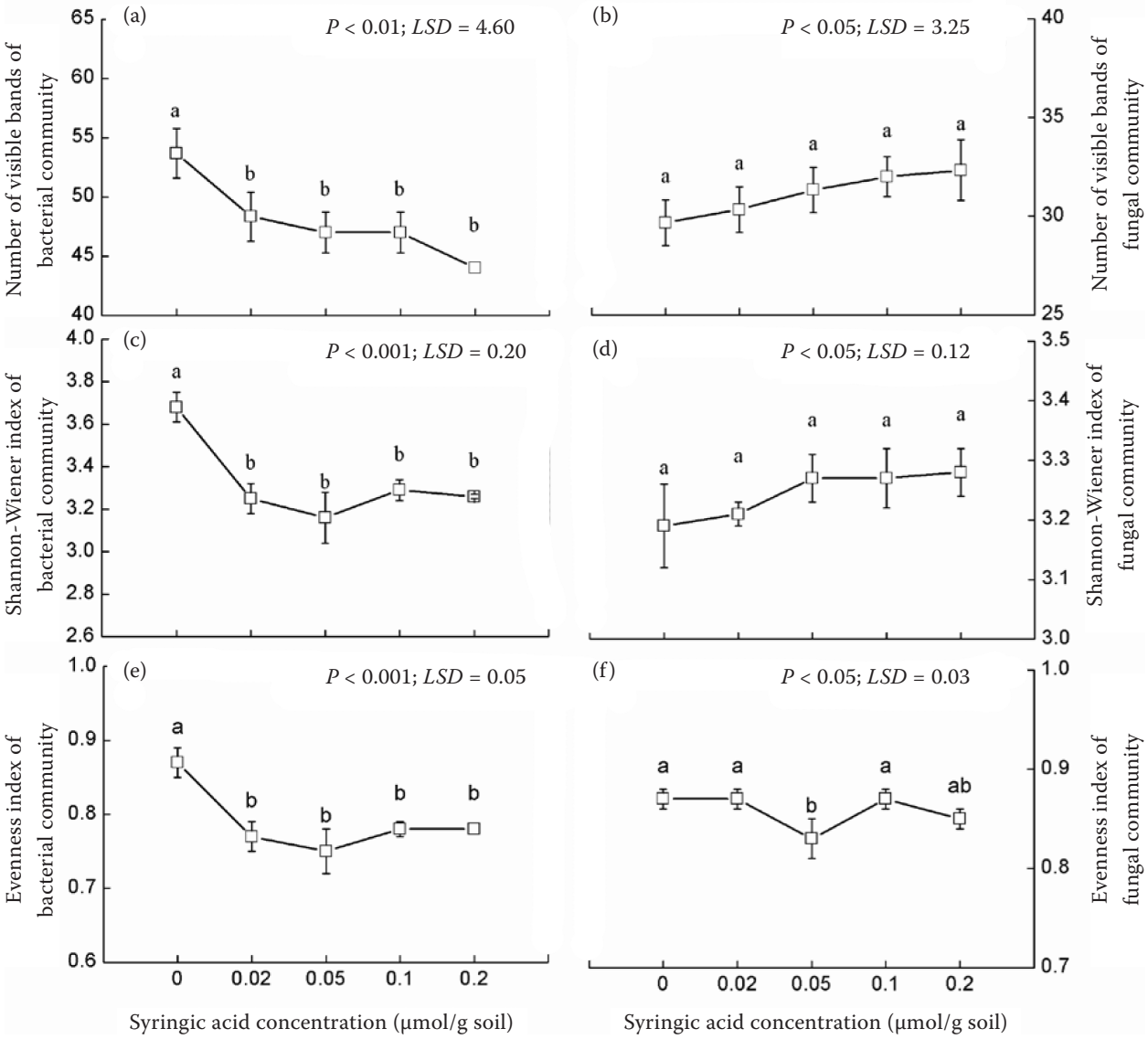

Figure 5. Diversity indices based on DGGE analysis of bacterial and fungal communities. Values (mean ± SE) with different letters are significantly different at the 0.05 probability level (Tukey's *HSD* test). *P* is from one-way ANOVA

1987). Therefore, in this study, SA was periodically added into the soil to maintain desired levels as described before (Blum et al. 1987). The physiological alterations caused by allelochemicals are often concentration dependent, and for many phenolic compounds the range of bioactivity is between 0.1 and 1 mmol/L (Piotrowski et al. 2008). The release rate of phenolic compounds from cucumber roots is about 0.01 μmol/day/plant in hydroponic solution (Yu and Matsui 1994). SA content in soils from long-term monocultured cucumber system was about 0.13 μmol/g soil (Zhou et al. 2012). Therefore, the total amount of SA used in this study was between 0.1 to 1.0 μmol/g soil, which was also within the realistic range of concentrations in the soil reported before (Piotrowski et al. 2008).

Blum et al. (2000) found that phenolic compounds inhibited cucumber seedling growth and stimulated culturable rhizosphere phenolic acid-utilizing (PAU) bacteria, and concluded that the inhibition of seedling growth and the simultaneous increase in PAU bacteria were not attributed to resource competition between the seedlings and the PAU bacteria. In this study, we also showed that rhizosphere soil dehydrogenase activity, MBC content, and bacterial and fungal community abundances were stimulated by SA. Moreover, SA decreased the bacteria-to-fungi ratio. These indicated that rhizosphere fungal community had a larger increase than rhizosphere bacterial community in response to SA, which is in line with the general viewpoint that phenolic-rich litter favors fungal-dominated food webs (Van Der Heijden et al. 2008).

Phenolic-related compounds have been shown to act as the predominant modulator of the soil bacterial communities (Badri et al. 2013). However, the reported effects of phenolic compounds on microorganism *in vitro* are mixed. SA was shown to have antifungal or antibacterial effects (Bais et al. 2005, Lanoue et al. 2010); while other study found it can stimulate the growth of *Fusarium oxysporum* (Wu et al. 2009). It is in the rhizosphere where soil microorganisms interact with plant roots (Inderjit 2005, Philippot et al. 2013). Thus, studies focused on the effects of phenolic compounds on specific microorganisms in the rhizosphere that are more ecologically realistic (Inderjit et al. 2009), which needs to be stressed in the future.

The rhizosphere microbial community is essential for plant functioning as it assists the plant in nutrient uptake and offers protection against pathogen attack (Van Der Heijden et al. 2008). Through releasing root exudates, plant can alter the soil microbial community structure and activity, which can have negative or positive feedbacks on plant growth (Kulmatiski et al. 2008). Therefore, it is possible that SA can affect cucumber growth indirectly, in part, by changing soil microbial communities. Future research should therefore focus on verifying the role of soil microbe-mediated plant-soil feedback in the toxic effects of phenolic compounds on cucumber.

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