PLOS ONE

RESEARCH ARTICLE

Changes in rhizosphere microbial communities in potted cucumber seedlings treated with syringic acid

Zhilin Wang[1], Jianhui Zhang[1], Fengzhi Wu[1,2], Xingang Zhou[1,2]*

1 Department of Horticulture, Northeast Agricultural University, Harbin, China, **2** Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (Northeast Region), Ministry of Agriculture, Harbin, China

* xingangzhou@yahoo.com

Citation: Wang Z, Zhang J, Wu F, Zhou X (2018) Changes in rhizosphere microbial communities in potted cucumber seedlings treated with syringic acid. PLoS ONE 13(6): e0200007. https://doi.org/10.1371/journal.pone.0200007

Editor: Krishnendu Acharya, University of Calcutta, INDIA

Received: January 21, 2018

Accepted: June 18, 2018

Published: June 28, 2018

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This work was supported by the National Natural Science Foundation of China (31772361), University Nursing Programme for Young Scholars with Creative Talents in Heilongjiang Province (UNPYSCT-2015002), 'Academic Backbone' Project of Northeast Agricultural University (17XG05), and China Agricultural Research System (CARS-23-C-10).

Abstract

Phytotoxic effects of phenolic compounds have been extensively studied, but less attention has been given to the effects of these compounds on soil microbial communities, which are crucial to the productivity of agricultural systems. Responses of cucumber rhizosphere bacterial and fungal communities to syringic acid (SA), a phenolic compound with autotoxicity to cucumber, were analyzed by high-throughput sequencing of 16S rRNA gene and internal transcribed spacer amplicons. SA at the concentration of 0.1 μmol g^{-1} soil changed rhizosphere bacterial and fungal community compositions, decreased bacterial community diversity but increased fungal community richness and diversity ($P<0.05$). Moreover, SA increased the relative abundances of bacterial phylum *Proteobacteria* and fungal classes *Leotiomycetes*, *Pezizomycetes*, *Tremellomycetes* and *Eurotiomycetes*, but decreased the relative abundances of bacterial phylum *Firmicutes* and fungal class *Sordariomycetes* ($P<0.05$). At the genus level, SA decreased the relative abundances of microbial taxa with pathogen-antagonistic and/or plant growth promoting potentials, such as *Pseudomonas* spp. ($P<0.05$). Real-time PCR validated that SA decreased cucumber rhizosphere *Pseudomonas* spp. abundance ($P<0.05$). *In vitro* study showed that SA (0.01 to 10 mM) inhibited the growth of a strain of *Pseudomonas* spp. with pathogen-antagonistic activities to cucumber pathogen *Fusarium oxysporum* f.sp. *cucumerinum* Owen ($P<0.05$). Overall, SA changed cucumber rhizosphere bacterial and fungal community compositions, which may exert negative effects on cucumber seedling growth through inhibiting plant-beneficial microorganisms.

Introduction

Modern agricultural systems, which are often characterized by short-term rotation or monocropping, usually decrease the plant diversity in the field [1]. The continuous monocropping system, in which the same crop is repeatedly monocropped on the same land, is not long-term sustainable because it usually decrease crop yield and quality, a phenomenon termed as 'soil sickness' [1,2]. Allelopathy is the suppression of the growth of one plant by another plant through the release of toxic chemical compounds (allelochemicals) into the environment [3,4].

Competing interests: The authors have declared that no competing interests exist.

Autotoxicity is a type of intra-specific allelopathy, where a plant species inhibits the growth of its own kind, and is supposed to be contributed to the soil sickness associated with several crops, such as asparagus (*Asparagus officinalis* L.), cucumber (*Cucumis sativus* L.), rice (*Oryza sativa* L.), sugarcane (*Saccharum officinalis* L.) and tea (*Camellia sinensis* L.) [5–7].

As an important class of plant secondary metabolites, phenolic compounds are suggested to be involved in plant-plant interactions and plant-microorganism interactions [7–10]. Several phenolic compounds, including derivatives of benzoic and cinnamic acids, have been identified from plant root exudates and decaying residues [6,11]. Mounting evidence shows that these compounds can have phytotoxic effects at proper concentrations, such as inhibiting plant nutrient ion uptake, enzyme activities and photosynthesis and respiration [3,6].

Soil microorganisms play critical roles in terrestrial ecosystems because they have profound influences on plant health and fitness, nutrient cycling, and decomposition of organic matter [12,13]. For example, many species of *Fusarium* are phytopathogenic fungi, which can cause diseases including vascular wilts and root rots [14]; while some *Pseudomonas* spp. can inhibit several plant pathogens and promote plant growth [15]. Previous studies demonstrated that phenolic compounds can affect microbial growth, biofilm formation, virulence and expression of genes encoding secondary metabolite production [16–18]. Moreover, phenolic compounds can have selective effects on microorganisms. For example, *p*-coumaric acid promoted the growth of *F. oxysporum* f.sp. *cucumerinum* Owen (FOC), a soil-borne plant pathogen of cucumber [19], while ferulic acid inhibited the growths of *P. fluorescens* and *Glomus intraradices* [18,20]. However, information about how phenolic compounds influence the whole soil microbial communities is still limited.

Soil sickness is one of the major constraints of cucumber production in the greenhouse [21,22]. Syringic acid (SA, 4-Hydroxy-3,5-dimethoxybenzoic acid) has been identified in cucumber-cultivated soils, and had inhibitory effects on cucumber seedling growth [22,23]. In a previous study, we found that SA changed cucumber rhizosphere bacterial and fungal community structures as evaluated by PCR & Denaturing Gradient Gel Electrophoresis [23]. However, detailed changes in rhizosphere microbial compositions are still unclear. In the present, we further analyzed cucumber rhizosphere bacterial and fungal communities with high-throughput amplicon sequencing technique, which can provide a higher resolution and a better understanding of environmental microbial communities than the PCR-based fingerprinting techniques [24,25]. Cucumber seedlings were treated with SA every other day. Rhizosphere microbial communities were analyzed ten days after the treatment. Moreover, cucumber rhizosphere *Pseudomonas* spp. abundance was estimated with real-time PCR assays. The effect of SA on the growth of a strain of *Pseudomonas* spp., a bacterium antagonistic to FOC, was also evaluated *in vitro*.

Materials and methods

Pot experiment

Soils were collected from the upper soil layer (0–15 cm) of an open field in the experimental station of Northeast Agricultural University, Harbin, China (45˚41'N, 126˚37'E), which was covered with grass and undisturbed for more than 15 years. No specific permissions were required for these locations/activities and the soil sampling did not involve endangered or protected species. The soil was a Mollisol soil with sandy loam texture, contained organic matter, 3.67%; available N, 89.02 mg kg^{-1}; Olsen P, 63.36 mg kg^{-1}; available K, 119.15 mg kg^{-1}; EC (1:2.5, w/v), 0.33 mS cm^{-1}; and pH (1:2.5, w/v), 7.78.

Cucumber seedlings (cv. Jinlv 3) with two cotyledons were planted into pots (10 cm diameter, 10 cm height) contained 150 g of soil. No fertilizer was used during the experiment. There

was one cucumber seedling per pot. Cucumber seedlings were maintained in a greenhouse (32˚C day/22˚C night, relative humidity of 60–80%, 16 h light/8 h dark).

Since phenolic acids could be rapidly metabolized by soil microorganisms after entering the soil [26], SA was applied into the soil periodically as suggested by Blum *et al.* [27]. At the one-leaf stage, cucumber seedlings were treated with SA at the concentration of 0.1 μmol g^{-1} soil every two days for five times. Seedlings treated with distilled water were used as the control. The solution pH was adjusted to 7.0 with 0.1 M sodium hydroxide solution because the soil pH is an important factor that regulates soil microbial communities [28]. Each treatment had five pots and was done in triplicate. Soil water content was adjusted every two days with distilled water to maintain the soil moisture at about 60% of its water holding capacity.

Soil sampling and DNA extraction

Ten days after the first treatment of SA, cucumber rhizosphere soils were collected from five seedlings in each replicate and were mixed to make a composite sample as described before [23]. There were three composite samples for each treatment and six composite samples in total.

Total soil DNA was extracted from 0.25 g soil with the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, USA) according to the manufacturer's instructions. Each composite soil sample was extracted in triplicate and the extracted DNA solutions were pooled. There were three composite DNA samples for each treatment.

Illumina Miseq sequencing and data processing

Cucumber rhizosphere bacterial and fungal community compositions were analyzed with high-throughput amplicon sequencing. The V3-V4 regions of the bacterial 16S rRNA gene and the ITS1 regions of the fungal rRNA gene were amplified with primer sets of F338/R806 and ITS1F/ITS2, respectively, as described before [29–31]. Both the forward and reverse primers also had a 6-bp barcode unique to each sample. Each DNA sample was independently amplified in triplicate, and the products of the triplicate PCR reactions were pooled and purified using the Agarose Gel DNA purification kit (TaKaRa, China). Then, purified amplicons were quantified by a TBS-380 micro fluorometer with Picogreen reagent (Invitrogen, USA), and mixed accordingly to achieve the equal concentration in the final mixture. The mixture was then paired-end sequenced (2×300) on an Illumina Miseq platform at Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China.

Raw sequence reads were de-multiplexed, quality-filtered, and processed using FLASH as described before [31,32]. Chimeric sequences were identified and removed using USEARCH 6.1 in QIIME [33]. Sequences were binned to Operational taxonomic units (OTUs) at 97% sequence similarity with USEARCH using an agglomerative clustering algorithm [34]. Then, a representative sequence of each OTU was taxonomically classified through BLAST against the SILVA [35] (bacteria) and Unite [36] (fungi) databases. The data set was deposited in the NCBI-Sequence Read Archive with the submission Accession Number SRP115338.

Real-time PCR analysis

Cucumber rhizosphere *Pseudomonas* spp. abundance was estimated with SYBR Green real-time PCR assays with primers of PsF/PsR [37] on an IQ5 real-time PCR system (Bio-Rad Lab, USA). The PCR protocol was: 95˚C for 5 min; followed by 30 cycles of 95˚C for 30 s, 65˚C for 30 s, 72˚C for 90 s; and a final extension at 72˚C for 10 min. Standard curves were made with a 10-fold dilution series (10^2–10^8) of plasmids containing 16S rRNA genes from soil samples. Sterile water was used as a negative control to replace the DNA template. All amplifications

were done in triplicate. The specificity of the products was confirmed by melting curve analysis and agarose gel electrophoresis. The threshold cycle (*Ct*) values obtained for each sample were compared with the standard curve to determine the initial copy number of the target gene.

In vitro experiment

Effects of SA on the growth of *Pseudomonas* spp. was studied *in vitro* with a microtiter plate-based assay method [38]. A strain of *Pseudomonas* spp. with antagonistic activity to FOC, *Pseudomonas* ZJH, was isolated and identified from greenhouse soils cultivated with tomato. *Pseudomonas* ZJH was grown aerobically in Luria-Bertani (LB) broth at 37˚C with shaking at 120 rpm overnight (in the log phase of growth). The culture was diluted to a final concentration of 1×10^6 cfu/ml with fresh LB medium supplemented with 0, 0.01, 0.1, 0.5, 1.0 and 10 mM of SA in 100 ml flasks. Then, the bacterial suspension was transferred into 96-well polystyrene microtiter plates with each well containing 200 μl of bacterial suspension. Microtiter plates were incubated at 37˚C with shaking at 120 rpm and the optical density at 600 nm (OD_{600}) in the wells was measured using a microtitre plate reader (Epoch, Biotek, USA) at 16 h. There were three microtiter wells for each treatments and the experiment was done in triplicate.

Statistical analysis

To avoid potential bias caused by sequencing depth, a random subsampling effort of 21,334 16S rRNA gene and 30,394 ITS gene sequences per sample was performed. The defined OTUs were used to calculate taxon accumulation curves with the 'vegan' package in 'R' (Version 3.3.1). For the alpha diversity analysis, Chao, ACE, Shannon index and inverse Simpson index were calculated using QIIME [33]. For the beta diversity analysis, weighted UniFrac distances and Bray-Curtis distances were calculated using QIIME [33] and 'vegan' package in 'R' (Version 3.3.1), respectively. Principal coordinates analysis was conducted to visualize the community similarity with the 'vegan' package in 'R' (Version 3.3.1). The shared and unique OTUs among treatments were counted, and their distributions were shown in a Venn diagram with the 'VennDiagram' package in 'R' (Version 3.3.1).

Data were analyzed by analysis of variance (ANOVA) in 'R' (Version 3.3.1). For data of alpha diversity indices and relative abundances of microbial taxa from Illumina Miseq sequencing analysis, and *Pseudomonas* spp. abundance from real-time PCR analysis, mean comparison between treatments was performed based on Welch's *t* test at the 0.05 probability level. For data of the growth of *Pseudomonas* spp. from the *in vitro* experiment, mean comparison between treatments was performed based on the Tukey's honestly significant difference (HSD) test at the 0.05 probability level.

Results

Sequence summary

After filtering reads by basal quality control and removing singleton OTUs, Illumina Miseq sequencing generated 24,696 quality bacterial sequences and 38,658 quality fungal sequences per sample on average. The average read lengths were 396 bp and 261 bp for the 16S rRNA genes and ITS regions, respectively. After clustering at the 97% sequence similarity, 1947 and 339 OTUs were identified for bacterial and fungal communities, respectively.

The Good's coverage of each soil sample, which reflects the captured diversity, was higher than 98% for bacterial community and higher than 99% for fungal community (data not shown). Rarefaction curves of OTUs at the 97% sequence similarity of all samples tended to approach the saturation plateau (S1 Fig), which also indicated that the sequencing depth was adequate.

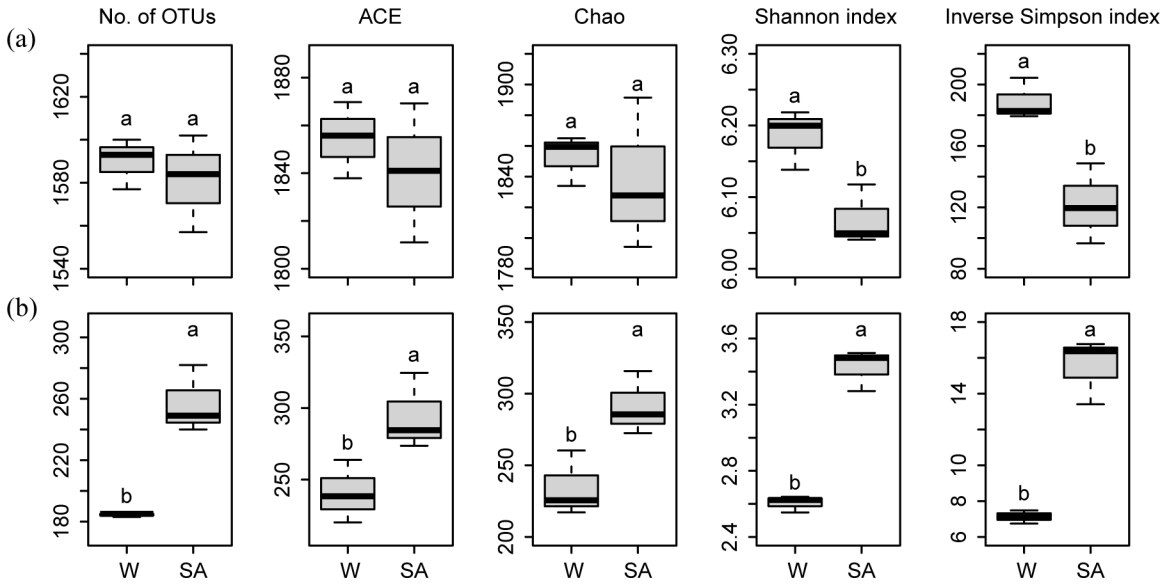


Fig 1. Diversity and richness indices of soil bacterial (a) and fungal (b) communities. OTUs were delineated at the 97% sequence similarity. These indices were calculated using random subsamples of 21,334 16S rRNA gene and 30,394 ITS gene sequences per sample. Different letters indicate significant difference based on Welch's *t* test (P<0.05). SA and W represent syringic acid- and water-treated soil samples, respectively.

https://doi.org/10.1371/journal.pone.0200007.g001

Alpha and beta diversities of bacterial and fungal communities

For bacterial communities, the number of OTUs, ACE and Chao indices did not significantly differ between the SA- and water-treated soil samples (Fig 1A). However, the Shannon index and inverse Simpson index were significantly lower in the SA-treated soil sample than in the water-treated soil sample (P<0.05).

For fungal communities, the number of OTUs, ACE, Chao, Shannon, and inverse Simpson indices were significantly higher in the SA-treated soil sample than in the water-treated soil sample (P<0.05) (Fig 1B).

For both bacterial and fungal communities, principal coordinates analysis based on Bray-Curtis and UniFrac distances resulted in a clear separation of SA- and water-treated soil samples along the first axis (Fig 2).

Bacterial community composition

Across all samples analyzed, a total of 27 bacterial phyla were detected, and 1.28% bacterial sequences were unclassified at the phylum level. The top three bacterial phyla were *Proteobacteria*, *Actinobacteria* and *Acidobacteria*, which had relative abundances ranging from 31.06% to 37.32%, from 19.28% to 23.27%, and from 12.96% to 14.37%, respectively (Fig 3A). These three bacterial phyla accounted for more than 68% of the bacterial sequences. *Chloroflexi*, *Firmicutes*, *Planctomycetes*, *Bacteroidetes* and *Gemmatimonadetes* were also detected at relatively high abundances (average relative abundance>1%). Compared with the water-treated soil sample, the SA-treated soil sample had significantly higher relative abundance of *Proteobacteria* and lower relative abundance of *Firmicutes* (P<0.05).

At the class level, all samples were dominated by *Actinobacteria*, *Acidobacteria*, *Betaproteobacteria*, *Alphaproteobacteria* and *Gammaproteobacteria* (average relative abundance>5%) (Fig 3B). These five bacterial classes accounted for more than 64% of the bacterial sequences. Compared with the water-treated soil sample, the SA-treated soil sample had significantly higher relative

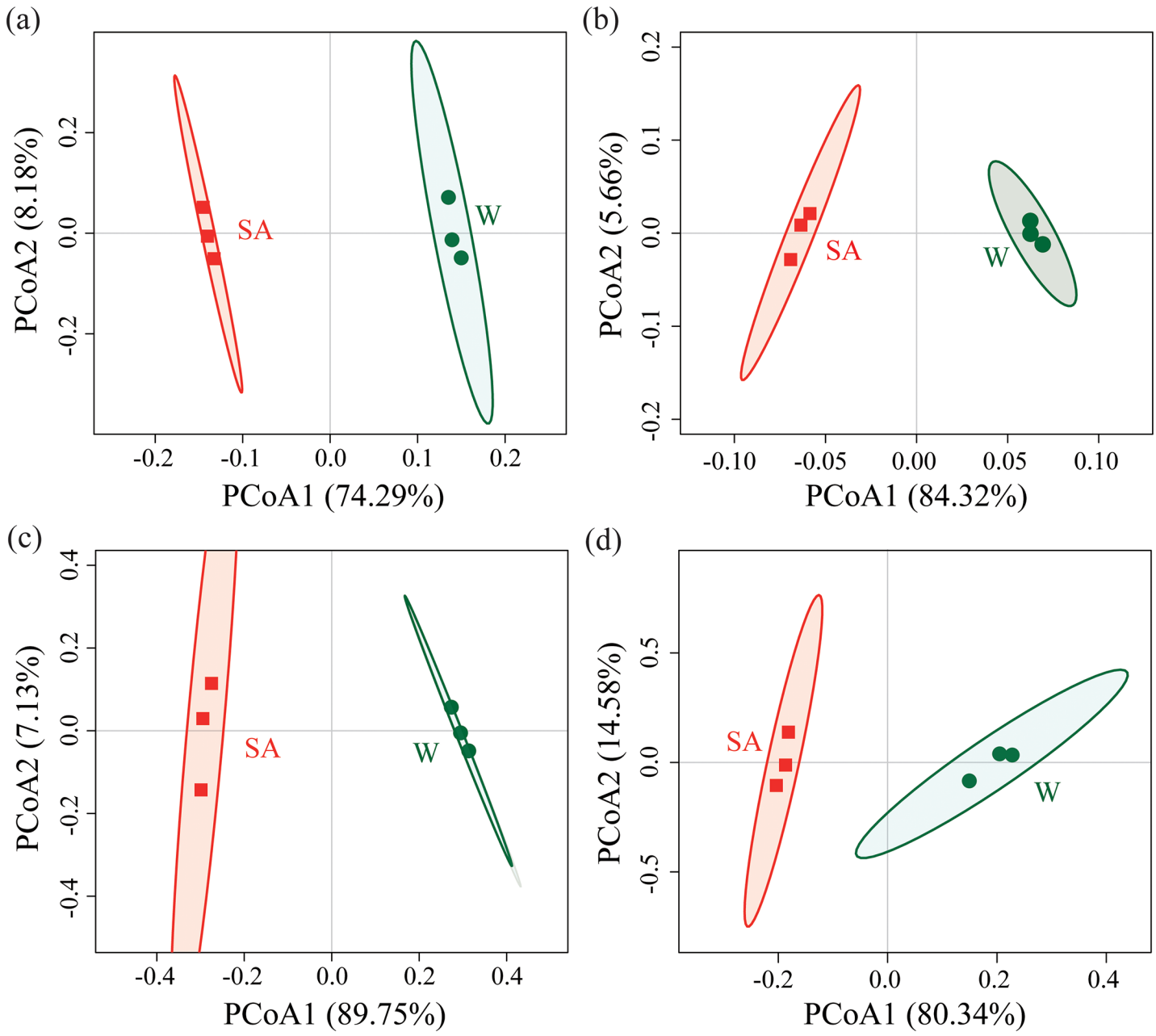


Fig 2. Beta diversities of bacterial and fungal communities. Differences in Bray-Curtis (a) and UniFrac distances (b) of bacterial communities, Bray-Curtis (c) and UniFrac distances (d) of fungal communities were visualized by principal component analyses. SA and W represent syringic acid- and water-treated soil samples, respectively. Ellipses indicate 95% confidence interval for replicates.

https://doi.org/10.1371/journal.pone.0200007.g002

abundances of *Betaproteobacteria* and *KD4-96*, but had lower relative abundances of *Gammaproteobacteria*, *Clostridia*, *Deltaproteobacteria*, *Cytophagia* and *Anaerolineae* ($P<0.05$).

At the genus level, compared with the water-treated soil sample, the SA-treated soil sample had significantly higher relative abundances of *Gaiella*, *Panacagrimonas*, *Blastococcus*, *Pisciniibacter* and *Azohydromonas* spp., but had lower relative abundances of *Clostridium sensu stricto 1*, *Steroidobacter*, *Acidibacter*, *Lysobacter*, *Terrisporobacter*, *Aeromicrobium*, *Pseudolabrys*, *Haliangium*, *Pseudomonas* and *Bradyrhizobium* spp. ($P<0.05$) (Figs 4A and 5A).

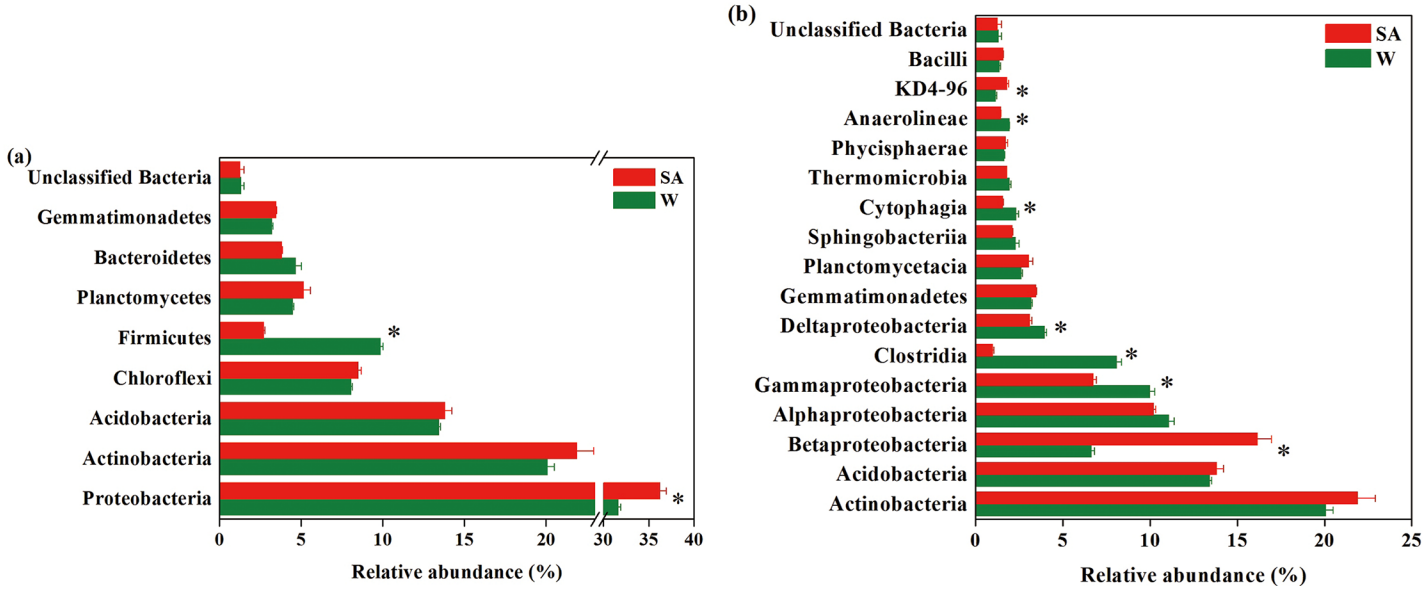


Fig 3. Relative abundances of main bacterial phyla (a) and classes (b) in the syringic acid (SA)- and water (W)-treated soil samples. Bacterial phyla and classes with average relative abundances >1% in at least one treatment were shown. Values are expressed as mean±standard error. Asterisks indicate significant difference between treatments based on Welch's *t* test (P<0.05).

https://doi.org/10.1371/journal.pone.0200007.g003

At the OTU level, the relative abundances of 12 dominant OTUs (average relative abundance>0.5%) were higher while 11 dominant OTUs were lower in SA-treated soil sample than in the water-treated soil sample (P<0.05) (S1 Table). These changed dominant OTUs mainly belonged to bacterial classes *Actinobacteria*, *Clostridia*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Acidobacteria*.

Fungal community composition

Fungal community was almost entirely composed of *Ascomycota* and *Zygomycota*, accounting for more than 95% of the fungal sequences (Fig 6A). Less abundant fungal phyla detected were *Basidiomycota* and *Chytridiomycota*. About 2.87% fungal sequences were not assigned to any known phylum. *Sordariomycetes*, *Leotiomycetes*, *Pezizomycetes* and *Zygomycetes* were the top four fungal classes, and they accounted for more than 92% of the fungal sequences (Fig 6B). Compared with the water-treated soil sample, the SA-treated soil sample had significantly higher relative abundances of phylum *Basidiomycota*, and classes *Leotiomycetes*, *Pezizomycetes*, *Tremellomycetes* and *Eurotiomycetes*, but had lower relative abundance of class *Sordariomycetes* (P<0.05).

The dominant families (average relative abundance>10%) in the SA-treated soil sample were *Chaetomiaceae* (18.91%), *Thelebolaceae* (18.55%), *Nectriaceae* (14.29%) and *Mortierellaceae* (14.08%), while these in the water-treated soil sample were *Chaetomiaceae* (31.32%), *Mortierellaceae* (19.62%) and *Lasiosphaeriaceae* (18.38%) (S2 Fig). More sequences were unclassified at the genus level in the water-treated soil sample than in the SA-treated soil sample for families *Chaetomiaceae* (29.93% vs. 10.55%), *Lasiosphaeriaceae* (17.97% vs. 3.25%) and *Mortierellaceae* (15.85% vs. 3.61%). Compared with the water-treated soil sample, the SA-treated soil sample had significantly higher relative abundances of families *Thelebolaceae*, *Tremellales incertae sedis*, *Gymnoascaceae*, and genera *Cryptococcus*, *Chaetomium*, *Kernia*, *Guehomyces* and *Wardomyces* spp., but had lower relative abundances of families *Chaetomiaceae* and *Lasiosphaeriaceae*, and genera *Pseudallescheria*, *Preussia* and *Chaetomidium* spp. (P<0.05) (Fig 4B and S2 Fig).

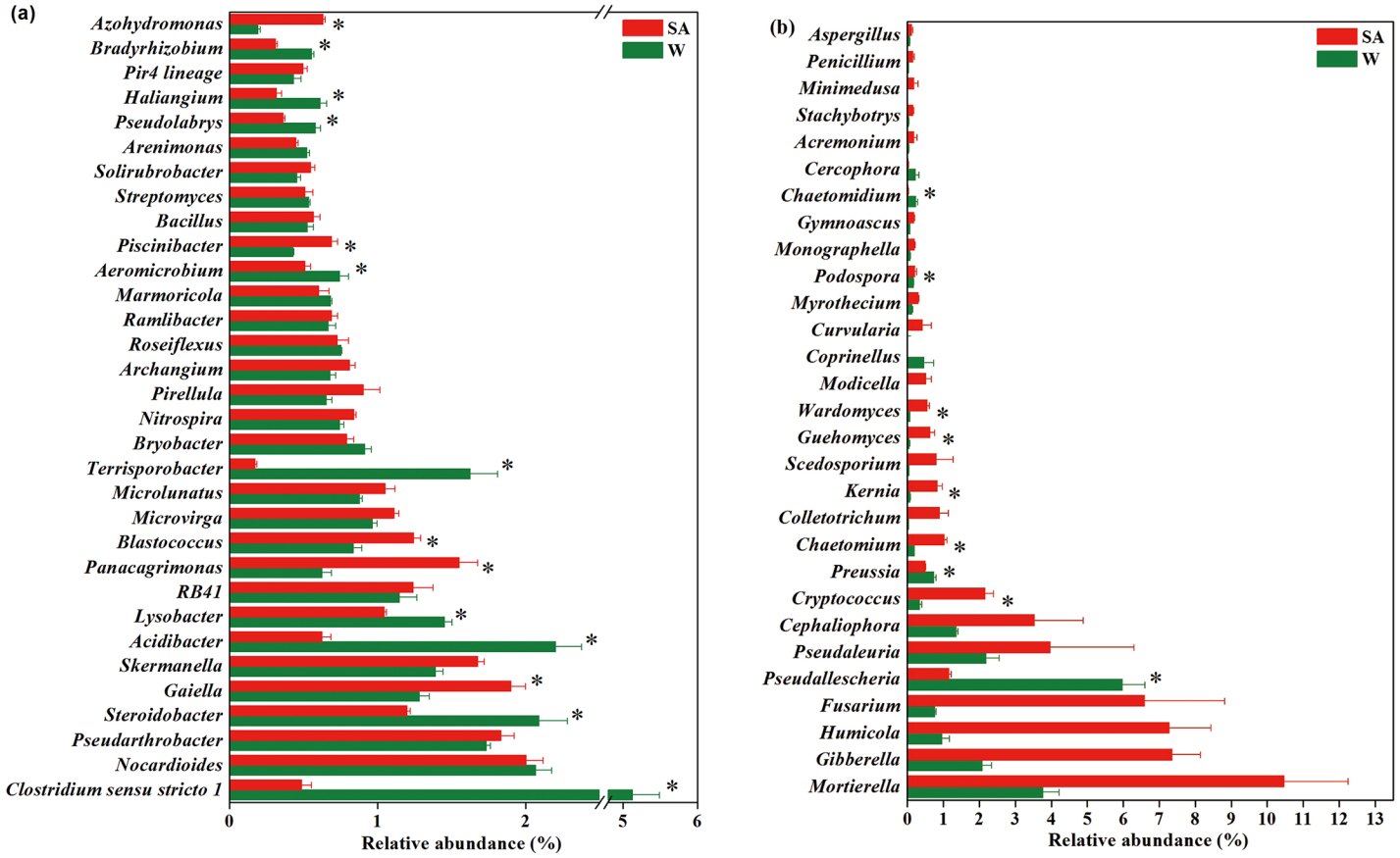


Fig 4. Relative abundances of main classified bacterial (a) and fungal genera (b) in the syringic acid (SA)- and water (W)-treated soil samples. Classified bacterial and fungal genera with average relative abundances >0.5% and 0.1%, respectively, in at least one treatment were shown. Values are expressed as mean±standard error. Asterisks indicate significant difference between treatments based on Welch's *t* test (P<0.05).

https://doi.org/10.1371/journal.pone.0200007.g004

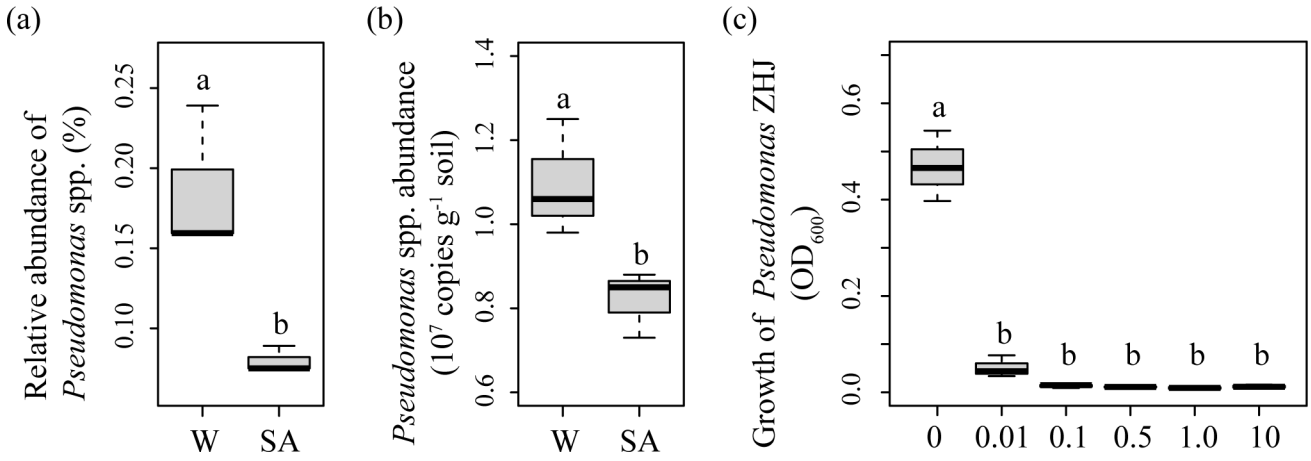


Fig 5. Effects of SA on the relative abundance of *Pseudomonas* spp. as estimated by Illumina Miseq sequencing (a), the abundance of *Pseudomonas* spp. as estimated by real-time PCR (b) and the growth of *Pseudomonas* ZJH *in vitro* (c). For (a) and (b), SA and W represent syringic acid- and water-treated soil samples, respectively. Different letters indicate significant difference based on Welch's *t* test (P<0.05). For (c), 0, 0.01, 0.1, 0.5, 1.0 and 10 represent the treatments of 0, 0.01, 0.1, 0.5 1.0 and 10 mM of syringic acid, respectively. Different letters indicate significant difference based on Tukey's HSD test (P<0.05).

https://doi.org/10.1371/journal.pone.0200007.g005

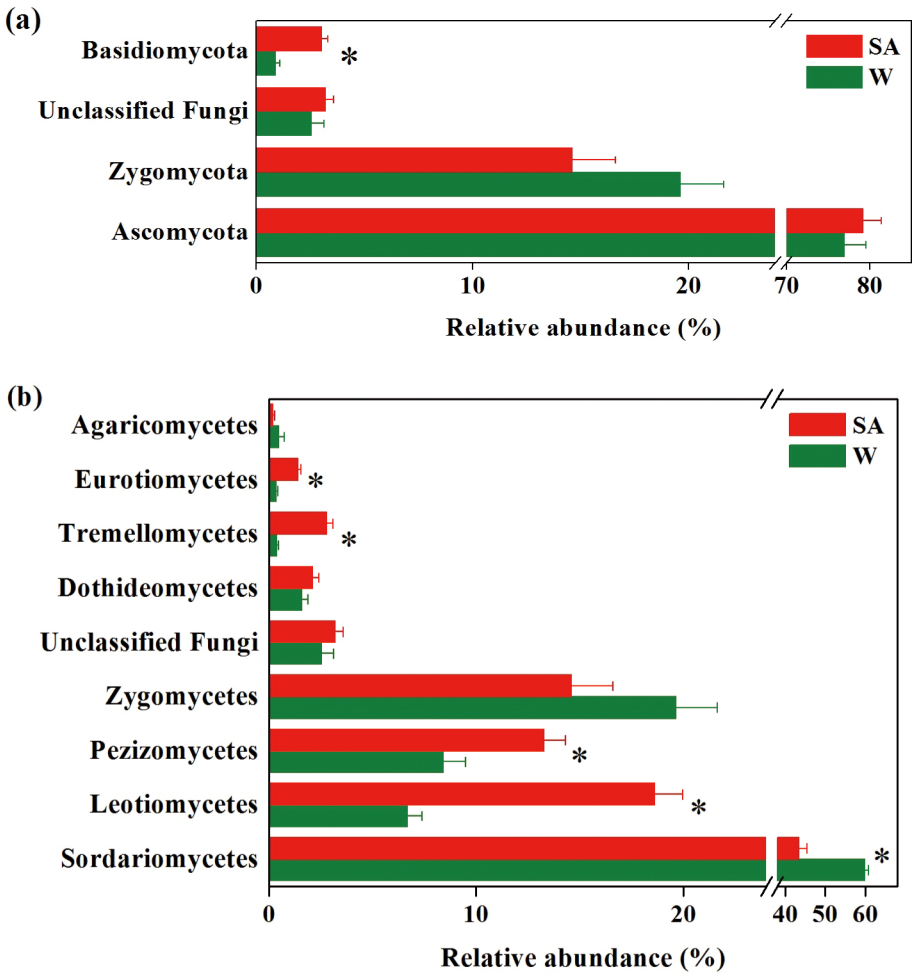


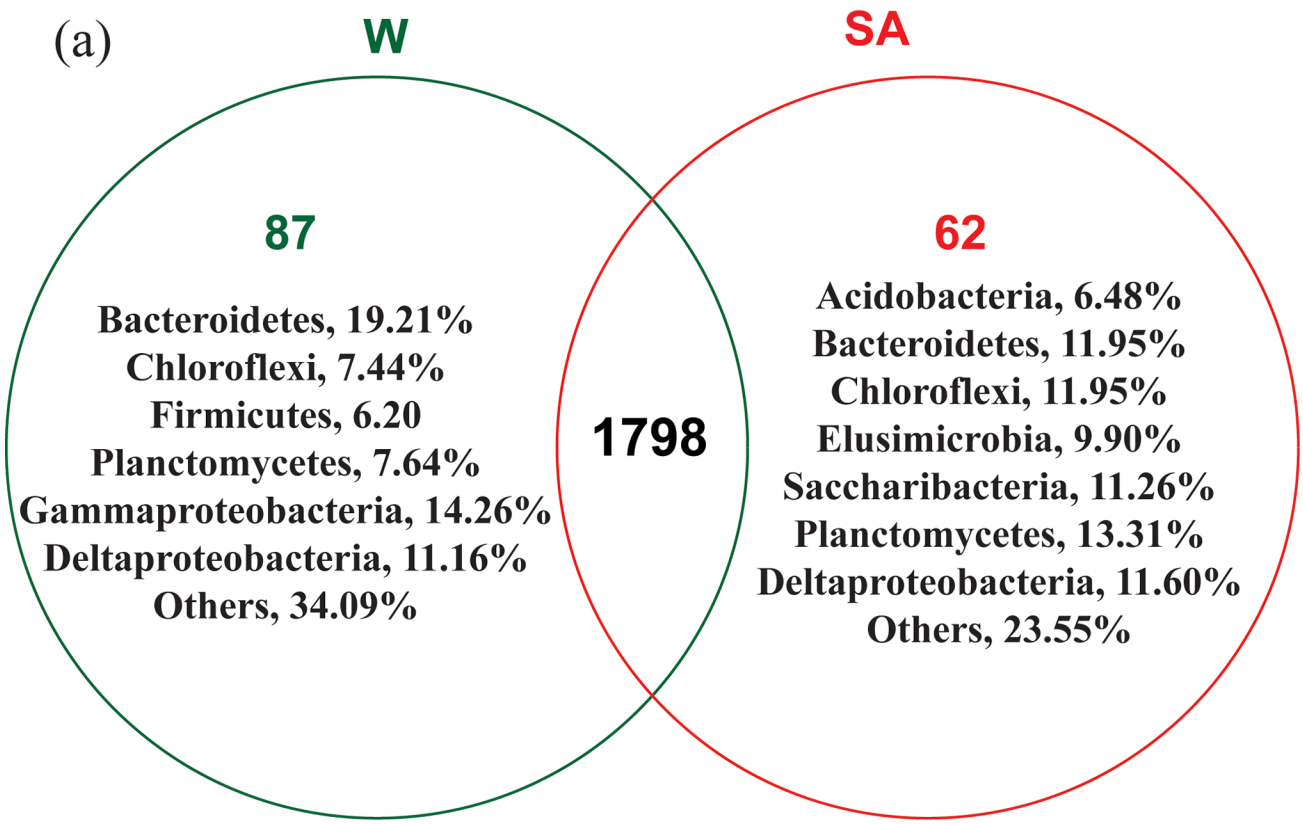
Fig 6. Relative abundances of main fungal phyla (a) and classes (b) in the syringic acid (SA)- and water (W)-treated soil samples. Fungal phyla and classes with average relative abundances >0.5% in at least one treatment were shown. Values are expressed as mean±standard error. Asterisks indicate significant difference between treatments based on Welch's *t* test (P<0.05).

https://doi.org/10.1371/journal.pone.0200007.g006

At the OTU level, the relative abundances of seven dominant OTUs (average relative abundance>0.5%), mainly composed of *Sordariomycetes* and *Zygomycetes*, were lower in SA-treated soil sample than in the water-treated soil sample (P<0.05) (S2 Table). The relative abundances of 27 dominant OTUs, mainly belonging to *Dothideomycetes*, *Eurotiomycetes*, *Leotiomycetes*, *Sordariomycetes*, *Tremellomycetes* and *Zygomycetes*, were higher in SA-treated soil sample than in the water-treated soil sample (P<0.05).

Shared and unique OTUs

For bacterial communities, SA- and water-treated soil samples shared 1798 OTUs, which accounted for 92.35% of the total bacterial OTUs observed (Fig 7A). Only a small proportion of OTUs were found to be unique to SA- or water-treated soil samples. OTUs unique to water-treated soil sample were dominated by sequences belonging to classes *Bacteroidetes*, *Gammaproteobacteria* and *Deltaproteobacteria*. OTUs unique to SA-treated soil sample were dominated by sequences belonging to classes *Planctomycetes*, *Bacteroidetes*, *Chloroflexi*, *Deltaproteobacteria* and *Saccharibacteria*.


(a)
W
SA
87
Bacteroidetes, 19.21%
Chloroflexi, 7.44%
Firmicutes, 6.20
Planctomycetes, 7.64%
Gammaproteobacteria, 14.26%
Deltaproteobacteria, 11.16%
Others, 34.09%
1798
62
Acidobacteria, 6.48%
Bacteroidetes, 11.95%
Chloroflexi, 11.95%
Elusimicrobia, 9.90%
Saccharibacteria, 11.26%
Planctomycetes, 13.31%
Deltaproteobacteria, 11.60%
Others, 23.55%

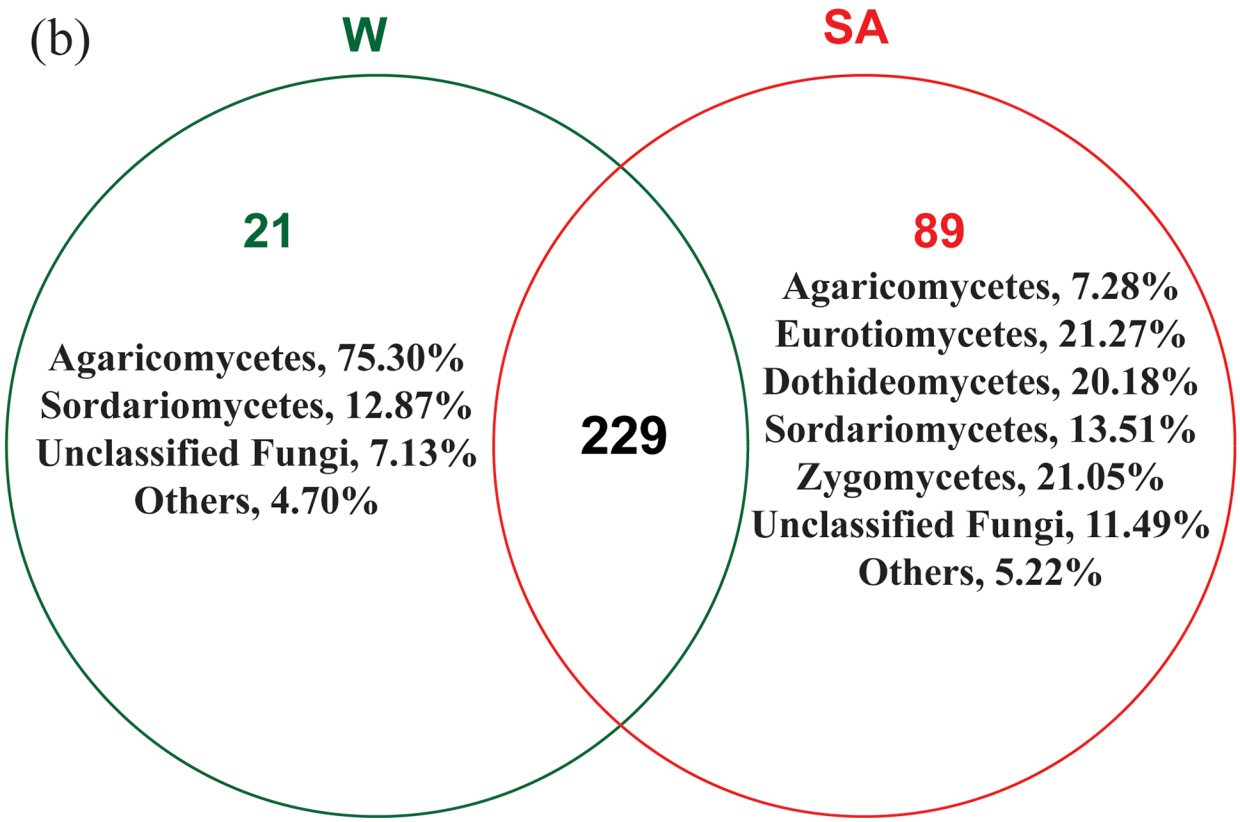

(b)
W
SA
21
Agaricomycetes, 75.30%
Sordariomycetes, 12.87%
Unclassified Fungi, 7.13%
Others, 4.70%
229
89
Agaricomycetes, 7.28%
Eurotiomycetes, 21.27%
Dothideomycetes, 20.18%
Sordariomycetes, 13.51%
Zygomycetes, 21.05%
Unclassified Fungi, 11.49%
Others, 5.22%

Fig 7. Venn diagrams demonstrating the numbers of shared and unique observed bacterial (a) and fungal (b) OTUs at 97% similarity between syringic acid (SA)- and water (W)-treated soil samples. Frequencies of OTUs unique to each treatment at the bacterial phylum/proteobacterial class and fungal class level were shown.

https://doi.org/10.1371/journal.pone.0200007.g007

For fungal communities, about 67.55% of total fungal OTUs were shared by all treatments. OTUs unique to water-treated soil sample were mainly composed of sequences belonging to *Agaricomycetes* at the class level and *Coprinellus* spp. at the genus level (72.70%); while these unique to SA-treated soil sample were dominated by sequences belonging to *Eurotiomycetes*, *Dothideomycetes* and *Zygomycetes* at the class level and *Modicella* (20.48%), Unclassified *Onygenaceae* (17.63%) and *Curvularia* spp. (15.61%) at the genus level (Fig 7B).

Cucumber rhizosphere *Pseudomonas* spp. abundance

Real-time PCR analysis showed that SA significantly decreased cucumber rhizosphere *Pseudomonas* spp. abundance (Fig 5B). The abundance of *Pseudomonas* spp. in the water-treated soil sample was about 1.34 times of that in the SA-treated soil sample.

Effects of SA on *Pseudomonas* ZJH *in vitro*

In vitro experiment showed that SA at the concentrations of 0.01 to 10 mM significantly inhibited the growth of *Pseudomonas* ZJH, which was antagonistic to the soil pathogen of cucumber FOC (Fig 5C). However, the effects of SA on the growth of *Pseudomonas* ZJH did not differ among different concentrations of SA.

Discussion

Autotoxicity is commonly acknowledged as one of the contributing factors to soil sickness, which severely threats sustainable agricultural production [6,22]. Soil microbial communities have profound effects on the growth, nutrition and health of plants in agricultural ecosystems [13]. In this study, we focused on the responses of rhizosphere microbial communities to SA, a phenolic compound with autotoxicity from cucumber. In natural ecosystems, soil SA concentration was shown to range from 0.05 to 0.1 μmol g^{-1} soil [39]. Previously, we also found that soil SA concentration in cucumber monocropping system ranged from 0.1 to 0.15 μmol g^{-1} soil [22] and exogenous SA at the concentrations of 0.05 to 0.2 μmol g^{-1} soil have detrimental effects on cucumber seedling growth [23]. Therefore, SA at 0.1 μmol g^{-1} soil was used in this study to simulate its effects on cucumber rhizosphere microbial communities in the monocropping system.

In the present study, the diversity indices of the bacterial communities (Shannon and inverse Simpson indices) were lower while these of the fungal communities were higher in the SA-treated soil sample than in the water-treated soil sample ($P<0.05$). Previously, our real-time PCR analysis showed that exogenous SA stimulated both bacterial and fungal community abundances with fungal community dad a larger increase [23]. These results suggested that bacterial and fungal communities responded differently to exogenous SA. This is not surprising considering that soil bacteria and fungi play different roles in degrading organic compounds. For example, fungi are generally regarded as main lignocellulose decomposers while bacteria prefer easily available carbon sources [40]. It has been observed that different microbial species have different abilities to degrade phenolic acids and also differ in their tolerances to the toxicity of phenolic acids [17,41,42]. Antagonistic interactions, through competition for substrate and production of antibiotics, occur between soil microorganisms [43]. The

increased fungal community diversity may be due to its less antagonism with bacterial community.

Principal coordinates analysis of the high-throughput amplicon sequencing data revealed that SA changed cucumber rhizosphere bacterial and fungal community structure, which confirmed our previous results of DNA fingerprinting analysis [23]. Our results also showed that the relative abundances of some taxa were higher while others were lower in SA-treated soil sample than in the water-treated soil sample, which was consistent with previous observations showing that plant root-released compounds had selective effects on soil microorganisms by promoting certain microorganisms and inhibiting others [44–46]. For example, phenolic compounds have been shown to promote the growth of *Fusarium* spp. [19,47] but inhibit the growth of *Azohydromonas* and *Pseudomonas* spp. at proper concentrations *in vitro* [48].

Microorganisms in soils are usually limited by the availability of carbon resources [12,44,49,50]. After entering soil, plant root released compounds can be assimilated by soil microorganisms [49]. In accordance with this notion, our SA-treated soil sample were enriched with several microbial taxa that were reported to be able to degrade simple phenolic compounds or other organic compounds containing aromatic rings, such as *Azohydromonas* [51], *Blastococcus* [52], *Chaetomium* [53], *Cryptococcus* [54] and *Guehomyces* [55] spp. Further studies should focus on validating the role of these microorganisms in degrading SA with techniques such as stable isotope probing.

Soil microorganisms can affect plant growth directly through forming mutualistic and pathogenic interactions, and indirectly through enhancing nutrient cycling as free-living ones [12,13]. Our Illumina Miseq sequencing results showed that SA-treated soil sample had significantly lower relative abundances of microbial taxa with plant-growth promoting potentials than in the water-treated soil sample. For example, species of *Pseudomonas* are known for their abilities to promote plant growth through phosphate solubilization and the production of phytohormones and volatile growth stimulants [56]. *Aeromicrobium* spp. can produce indole-3-acetic acid (IAA) and solubilize phosphate [57]. *Bradyrhizobium* spp. contains strains with high phosphate solubilizing, IAA and siderophore producing activities, and can promote the growth of radish (*Raphanus sativus* L.) [58]. Some *Preussia* spp. have plant growth-promoting activities through releasing IAA [59,60].

Microbial taxa that had plant pathogen-inhibiting potentials or may be related to soil suppressiveness to soil-borne pathogens were inhibited by SA. For example, *Haliangium*, *Lysobacter*, *Pseudomonas* and *Preussia* spp. can produce secondary metabolites to inhibit plant fungal pathogens [61,62]. Species of *Chaetomidium* spp. inhibited the growth of *Gaeumannomyces graminis* var. *tritici*, a root pathogen of barley [63]. *Aeromicrobium* spp. was higher in potato common scab-suppressive soil [64]. *Steroidobacter* spp. was higher in the rhizosphere of healthy *Lilium davidii* var. *unicolor* than in the *Fusarium*-wilted ones [65]. Soil that was suppressive to Panama disease of banana had higher *Pseudolabrys* spp. [66]. Both Illumina Miseq sequencing and real-time PCR showed that SA had inhibitory effect on *Pseudomonas* spp. in cucumber rhizosphere. *In vitro* experiment also confirmed that SA inhibited the growth of a strain of *Pseudomonas* spp. with antagonistic activity to FOC. Therefore, the inhibition of plant beneficial microbes by phenolic compounds may be linked to the retarded plant growth and accumulation of soil-borne plant pathogens that observed in long-term monocropping systems [1,2,31].

Conclusions

In summary, we showed that SA changed cucumber rhizosphere bacterial and fungal community compositions, decreased the bacterial community diversity but increased fungal

community richness and diversity. Moreover, SA have selective effects on soil microorganisms by promoting certain microorganisms and inhibiting others. Importantly, SA decreased the relative abundances of several microbial taxa with plant-growth promoting or plant pathogen-inhibiting potentials. Real-time PCR analysis and *in vitro* experiment validated that SA had inhibitory effects on *Pseudomonas* spp. Plant-released compounds play an important role in the complex interactions that occur between plants and soil microorganisms, and among soil microorganisms [46,49,50]. Further investigations are required to elucidate the interactions among cucumber, *Pseudomonas* spp. and soil-borne pathogens (e.g., FOC) mediated by SA.

Supporting information

S1 Fig. Rarefaction curves of the number of operational taxonomic units (OTUs) for bacterial (a) and fungal communities (b) in each sample. Random subsamples of 21,334 16S rRNA gene and 30,394 ITS gene sequences per sample were used to generate the rarefaction curves. OTUs were delineated at the 97% sequence similarity.
(TIF)

S2 Fig. Relative abundances of fungal families in the syringic acid (SA)- and water (W)-treated soil samples. Fungal families with average relative abundances >1% (a) and >0.1% (b) in at least one treatment were shown. Values are expressed as mean±standard error. Asterisks indicate significant difference between treatments based on Welch's *t* test ($P<0.05$).
(TIF)

S1 Table. The most abundant bacterial OTUs in the syringic acid (SA)- and water (W)-treated soil samples. OTUs were delineated at the 97% sequence similarity. Only OTUs with average relative abundances >0.5% in at least one treatment were presented. Values were expressed as mean±standard error. OTU ID in bold indicates its relative abundance was significant different between treatments according to Welch's *t* test ($P<0.05$).
(DOC)

S2 Table. The most abundant fungal OTUs in the syringic acid (SA)- and water (W)-treated soil samples. OTUs were delineated at the 97% sequence similarity. Only OTUs with average relative abundances >0.1% in at least one treatment were presented. Values were expressed as mean±standard error. OTU ID in bold indicates its relative abundance was significant different between treatments according to Welch's *t* test ($P<0.05$).
(DOC)

Author Contributions

Conceptualization: Fengzhi Wu, Xingang Zhou.

Data curation: Zhilin Wang, Xingang Zhou.

Funding acquisition: Fengzhi Wu, Xingang Zhou.

Investigation: Zhilin Wang, Jianhui Zhang, Xingang Zhou.

Resources: Xingang Zhou.

Writing – original draft: Xingang Zhou.

References

1. Cook RJ. Toward cropping systems that enhance productivity and sustainability. Proc Nat Acad Sci USA. 2006; 103: 18389–18394. https://doi.org/10.1073/pnas.0605946103 PMID: 17130454

2. van der Putten WH, Bardgett RD, Bever JD, Bezemer TM, Casper BB, Fukami T, et al. Plant–soil feedbacks: the past, the present and future challenges. J Ecol. 2013; 101: 265–276.
3. Scavo A, Restuccia A, Mauromicale G. Allelopathy: principles and basic aspects for agroecosystem control. Sustain Agric Rev. 2018; 28: 47–101.
4. Trezzi MM, Vidal RA, Balbinot AA, Bittencourt HV, Souza A. Allelopathy: driving mechanisms governing its activity in agriculture. J Plant Interact. 2016; 11: 53–60.
5. Jia XL, Ye JH, Zhang Q, Li L, Hu YL, Zheng MZ, et al. Soil toxicity and microbial community structure of Wuyi rock tea plantation. Allelopathy J. 2017; 41: 113–126.
6. Singh HP, Batish DR, Kohli RK. Autotoxicity: Concept, organisms and ecological significance. Crit Rev Plant Sci. 1999; 18: 757–772.
7. Zhou X, Zhang J, Pan D, Ge X, Jin X, Chen S, Wu F. *p*-Coumaric can alter the composition of cucumber rhizosphere microbial communities and induce negative plant-microbial interactions. Biol Fert Soils. 2018; 54: 363–372.
8. Kaur H, Kaur R, Kaur S, Baldwin IT, Inderjit. Taking ecological function seriously: soil microbial communities can obviate allelopathic effects of released metabolites. PloS one. 2009; 4: e4700. https://doi.org/10.1371/journal.pone.0004700 PMID: 19277112
9. Pierik R, Mommer L, Voesenek L. Molecular mechanisms of plant competition: neighbour detection and response strategies. Funct Ecol. 2013; 27: 841–853.
10. Politycka B, Smolen S, Golcz A, Lukaszewicz S. Effects of selenium on contents of selected micronutrients and activities of antioxidant enzymes in cucumber roots exposed to *p*-coumaric acid. Allelopathy J. 2017; 41: 101–112.
11. Yu JQ, Matsui Y. Phytotoxic substances in the root exudates of *Cucumis sativus* L. J Chem Ecol. 1994; 20: 21–31. https://doi.org/10.1007/BF02065988 PMID: 24241696
12. Bardgett RD, van der Putten WH. Belowground biodiversity and ecosystem functioning. Nature. 2014; 515: 505–511. https://doi.org/10.1038/nature13855 PMID: 25428498
13. Bender SF, Wagg C, van der Heijden MGA. An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. Trends Ecol Evol. 2016; 31: 440–452. https://doi.org/10.1016/j.tree.2016.02.016 PMID: 26993667
14. Alabouvette C, Olivain C, Migheli Q, Steinberg C. Microbiological control of soil-borne phytopathogenic fungi with special emphasis on wilt-inducing *Fusarium oxysporum*. New Phytol. 2009; 184: 529–544. https://doi.org/10.1111/j.1469-8137.2009.03014.x PMID: 19761494
15. Weyens N, van der Lelie D, Taghavi S, Newman L, Vangronsveld J. Exploiting plant-microbe partnerships to improve biomass production and remediation. Trends Biotechnol. 2009; 27: 591–598. https://doi.org/10.1016/j.tibtech.2009.07.006 PMID: 19683353
16. de Werra P, Huser A, Tabacchi R, Keel C, Maurhofer M. Plant- and microbe-derived compounds affect the expression of genes encoding antifungal compounds in a Pseudomonad with biocontrol activity. Appl Environ Microbiol. 2011; 77: 2807–2812. https://doi.org/10.1128/AEM.01760-10 PMID: 21357434
17. Sánchez-Maldonado AF, Schieber A, Gänzle MG. Structure-function relationships of the antibacterial activity of phenolic acids and their metabolism by lactic acid bacteria. J Appl Microbiol. 2011; 111: 1176–1184. https://doi.org/10.1111/j.1365-2672.2011.05141.x PMID: 21895894
18. Lemos M, Borges A, Teodosio J, Araujo P, Mergulhao F, Melo L, et al. The effects of ferulic and salicylic acids on *Bacillus cereus* and *Pseudomonas fluorescens* single- and dual-species biofilms. Int Biodeter Biodegrad. 2014; 86: 42–51.
19. Zhou X, Wu F. *p*-Coumaric acid influenced cucumber rhizosphere soil microbial communities and the growth of *Fusarium oxysporum* f. sp. *cucumerinum* Owen. PloS one. 2012; 7: e48288. https://doi.org/10.1371/journal.pone.0048288 PMID: 23118972
20. Medina A, Jakobsen I, Egsgaard H. Sugar beet waste and its component ferulic acid inhibits external mycelium of arbuscular mycorrhizal fungus. Soil Biol Biochem. 2011; 43: 1456–1463.
21. Zhou X, Yu G, Wu F. Effects of intercropping cucumber with onion or garlic on soil enzyme activities, microbial communities and cucumber yield. Eur J Soil Biol. 2011; 47: 279–287.
22. Zhou X, Yu G, Wu F. Soil phenolics in a continuously mono-cropped cucumber (*Cucumis sativus* L.) system and their effects on cucumber seedling growth and soil microbial communities. Eur J Soil Sci. 2012; 63: 332–340.
23. Zhou X, Wu F, Xiang W. Syringic acid inhibited cucumber seedling growth and changed rhizosphere microbial communities. Plant Soil Environ. 2014; 60: 158–164.
24. Schöler A, Jacquiod S, Vestergaard G, Schulz S, Schloter M. Analysis of soil microbial communities based on amplicon sequencing of marker genes. Biol Fert Soils. 2017; 53: 485–489.

25. Vestergaard G, Schulz S, Schöler A, Schloter M. Making big data smart-how to use metagenomics to understand soil quality. Biol Fert Soils. 2017; 53: 479–484.
26. Shafer SR, Blum U. Influence of phenolic acids on microbial populations in the rhizosphere of cucumber. J Chem Ecol. 1991; 17: 369–389. https://doi.org/10.1007/BF00994339 PMID: 24258732
27. Blum U, Weed SB, Dalton BR. Influence of various soil factors on the effects of ferulic acid on leaf expansion of cucumber seedlings. Plant Soil. 1987; 98: 111–130.
28. Fierer N, Jackson RB. The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci USA. 2006; 103: 626–631. https://doi.org/10.1073/pnas.0507535103 PMID: 16407148
29. Crowther TW, Maynard DS, Leff JW, Oldfield EE, McCulley RL, Fierer N, et al. Predicting the responsiveness of soil biodiversity to deforestation: a cross-biome study. Global Change Biol. 2014; 20: 2983–2994.
30. Derakhshani H, Tun HM, Khafipour E. An extended single-index multiplexed 16S rRNA sequencing for microbial community analysis on MiSeq illumina platforms. J Basic Microb. 2016; 56: 321–326.
31. Zhou X, Liu J, Wu F. Soil microbial communities in cucumber monoculture and rotation systems and their feedback effects on cucumber seedling growth. Plant Soil. 2017; 415: 507–520.
32. Magoc T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics. 2011; 27: 2957–2963. https://doi.org/10.1093/bioinformatics/btr507 PMID: 21903629
33. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010; 7: 335–336. https://doi.org/10.1038/nmeth.f.303 PMID: 20383131
34. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010; 26: 2460–2641. https://doi.org/10.1093/bioinformatics/btq461 PMID: 20709691
35. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucl Acids Res. 2013; 41: D590–D596. https://doi.org/10.1093/nar/gks1219 PMID: 23193283
36. Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, et al. Towards a unified paradigm for sequence-based identification of fungi. Mol Ecol. 2013; 22: 5271–5277. https://doi.org/10.1111/mec.12481 PMID: 24112409
37. Garbeva P, van Veen JA, van Elsas JD. Assessment of the diversity, and antagonism towards *Rhizoctonia solani* AG3, of *Pseudomonas* species in soil from different agricultural regimes. FEMS Microbiol Ecol. 2004; 47: 51–64. https://doi.org/10.1016/S0168-6496(03)00234-4 PMID: 19712346
38. Casey JT, O'Cleirigh C, Walsh PK, O'Shea DG. Development of a robust microtiter plate-based assay method for assessment of bioactivity. J Microbiol Meth. 2004; 58: 327–334.
39. Muscolo A, Sidari M. Seasonal fluctuations in soil phenolics of a coniferous forest: effects on seed germination of different coniferous species. Plant Soil. 2006; 284: 305–318.
40. de Boer W, Folman LB, Summerbell RC, Boddy L. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiol Rev. 2005; 29: 795–811. https://doi.org/10.1016/j.femsre.2004.11.005 PMID: 16102603
41. Zhou X, Wang Z, Pan D, Wu F. Effects of vanillin on cucumber (*Cucumis sativus* L.) seedling rhizosphere *Bacillus* and *Pseudomonas* spp. community structures. Allelopathy J. 2018, 43: 255–264.
42. Wu H, Xu J, Wang J, Qin X, Wu L, Li Z, et al. Insights into the mechanism of proliferation on the special microbes mediated by phenolic acids in the *Radix pseudostellariae* rhizosphere under continuous monoculture regimes. Front Plant Sci. 2017; 8:659. https://doi.org/10.3389/fpls.2017.00659 PMID: 28512464
43. Meidute S, Demoling F, Bååth E. Antagonistic and synergistic effects of fungal and bacterial growth in soil after adding different carbon and nitrogen sources. Soil Biol Biochem. 2008; 40: 2334–2343.
44. Badri DV, Chaparro JM, Zhang R, Shen Q, Vivanco JM. Application of natural blends of phytochemicals derived from the root exudates of *Arabidopsis* to the soil reveal that phenolic-related compounds predominantly modulate the soil microbiome. J Biol Chem. 2013; 288: 4502–4512. https://doi.org/10.1074/jbc.M112.433300 PMID: 23293028
45. Liu J, Li X, Jia Z, Zhang T, Wang X. Effect of benzoic acid on soil microbial communities associated with soilborne peanut diseases. Appl Soil Ecol. 2017; 110: 34–42.
46. Yuan H, Zhu Z, Liu S, Ge T, Jing H, Li B, et al. Microbial utilization of rice root exudates: ^{13}C labeling and PLFA composition. Bio Fert Soils. 2016; 52: 615–627.
47. Lanoue A, Burlat V, Henkes GJ, Koch I, Schurr U, Röse USR. *De novo* biosynthesis of defense root exudates in response to *Fusarium* attack in barley. New Phytol. 2010; 185: 577–588. https://doi.org/10.1111/j.1469-8137.2009.03066.x PMID: 19878462

48. Dietrich D, Illman B, Crooks C. Differential sensitivity of polyhydroxyalkanoate producing bacteria to fermentation inhibitors and comparison of polyhydroxybutyrate production from *Burkholderia cepacia* and *Pseudomonas pseudoflava*. BMC Res Notes. 2013; 6: 219. https://doi.org/10.1186/1756-0500-6-219 PMID: 23734728

49. Sasse J, Martinoia E, Northen T. Feed your friends: do plant exudates shape the root microbiome? Trends Plant Sci. 2018; 23: 25–41. https://doi.org/10.1016/j.tplants.2017.09.003 PMID: 29050989

50. Ge T, Li B, Zhu Z, Hu Y, Yuan H, Dorodnikov M, et al. Rice rhizodeposition and its utilization by microbial groups depends on N fertilization. Biol Fert Soils. 2017; 53: 37–48.

51. Song Y, Li Y, Zhang W, Wang F, Bian YR, Boughner LA, et al. Novel biochar-plant tandem approach for remediating hexachlorobenzene contaminated soils: proof-of-concept and new insight into the rhizosphere. J Agr Food Chem. 2016; 64: 5464–5471.

52. Li R, Khafipour E, Krause DO, Entz MH, de Kievit TR, Fernando WGD. Pyrosequencing reveals the influence of organic and conventional farming systems on bacterial communities. PloS one. 2012; 7: e51897. https://doi.org/10.1371/journal.pone.0051897 PMID: 23284808

53. Haider K, Trojanowski J. Decomposition of specifically ^{14}C-labelled phenols and dehydropolymers of coniferyl alcohol as models for lignin degradation by soft and white rot fungi. Arch Microbiol. 1975; 105: 33–41.

54. DeRito CM, Madsen EL. Stable isotope probing reveals *Trichosporon* yeast to be active *in situ* in soil phenol metabolism. ISME J. 2009; 3: 477–485. https://doi.org/10.1038/ismej.2008.122 PMID: 19092862

55. Fernandez PM, Martorell MM, Blaser MG, Ruberto LAM, de Figueroa LIC, Mac Cormack W. Phenol degradation and heavy metal tolerance of Antarctic yeasts. Extremophiles. 2017; 21: 445–457. https://doi.org/10.1007/s00792-017-0915-5 PMID: 28271165

56. Rahmoune B, Morsli A, Khelifi-Slaoui M, Khelifi L, Strueh A, Erban A, et al. Isolation and characterization of three new PGPR and their effects on the growth of *Arabidopsis* and *Datura* plants. J Plant Interact. 2017; 12: 1–6.

57. Yadav AN, Sachan SG, Verma P, Saxena AK. Prospecting cold deserts of north western Himalayas for microbial diversity and plant growth promoting attributes. J Biosci Bioeng. 2015; 119: 683–693. https://doi.org/10.1016/j.jbiosc.2014.11.006 PMID: 25575970

58. Antoun H, Beauchamp CJ, Goussard N, Chabot R, Lalande R. Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: Effect on radishes (*Raphanus sativus* L.). Plant Soil. 1998; 204: 57–67.

59. Khan AL, Al-Harrasi A, Al-Rawahi A, Al-Farsi Z, Al-Mamari A, Waqas M, et al. Endophytic fungi from Frankincense tree improves host growth and produces extracellular enzymes and indole acetic acid. Plos One. 2016; 11: e0158207. https://doi.org/10.1371/journal.pone.0158207 PMID: 27359330

60. Mapperson RR, Kotiw M, Davis RA, Dearnaley JD. The diversity and antimicrobial activity of *Preussia* sp. endophytes isolated from Australian dry rainforests. Curr Microbiol. 2014; 68: 30–37. https://doi.org/10.1007/s00284-013-0415-5 PMID: 23975673

61. Expósito RG, Postma J, Raaijmakers JM, De Bruijn I. Diversity and activity of *Lysobacter* species from disease suppressive soils. Front Microbiol. 2015; 6: 1243. https://doi.org/10.3389/fmicb.2015.01243 PMID: 26635735

62. Fudou R, Iizuka T, Yamanaka S. Haliangicin, a novel antifungal metabolite produced by a marine myxobacterium. J Antibiot. 2001; 54: 149–152. PMID: 11302487

63. Macia-Vicente JG, Jansson HB, Mendgen K, Lopez-Llorca LV. Colonization of barley roots by endophytic fungi and their reduction of take-all caused by *Gaeumannomyces graminis* var. *tritici*. Can J Microbiol. 2008; 54: 600–609. https://doi.org/10.1139/w08-047 PMID: 18772922

64. Rosenzweig N, Tiedje JM, Quensen JF, Meng QX, Hao JJJ. Microbial communities associated with potato common scab-suppressive soil determined by pyrosequencing analyses. Plant Dis. 2012; 96: 718–725.

65. Shang QH, Yang G, Wang Y, Wu XK, Zhao X, Hao HT, et al. Illumina-based analysis of the rhizosphere microbial communities associated with healthy and wilted Lanzhou lily (*Lilium davidii* var. *unicolor*) plants grown in the field. World J Microbiol Biotechnol. 2016; 32: 95. https://doi.org/10.1007/s11274-016-2051-2 PMID: 27116961

66. Xue C, Penton CR, Shen ZZ, Zhang RF, Huang QW, Li R, et al. Manipulating the banana rhizosphere microbiome for biological control of Panama disease. Sci Rep. 2015; 5: 11124. https://doi.org/10.1038/srep11124 PMID: 26242751