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ORIGINAL PAPER

Pathogen invasion indirectly changes the composition of soil microbiome via shifts in root exudation profile

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Abstract Plant-derived root exudates modulate plant-microbe interactions and may play an important role in pathogen suppression. Root exudates may, for instance, directly inhibit pathogens or alter microbiome composition. Here, we tested if plants modulate their root exudation in the presence of a pathogen and if these shifts alter the rhizosphere microbiome composition. We added exudates from healthy and *Ralstonia solanacearum*-infected tomato plants to an unplanted soil and followed changes in bacterial community composition. The presence of pathogen changed the exudation of phenolic compounds and increased the release of caffeic acid. The amendment of soils with exudates from the infected plants led to a development of distinct and less diverse soil microbiome communities. Crucially, we could reproduce similar shift in microbiome composition by adding pure caffeic acid into the soil. Caffeic acid further suppressed *R. solanacearum* growth *in vitro*. We conclude that pathogen-induced changes in root exudation profile may serve to control pathogen both by direct inhibition and by indirectly shifting the composition of rhizosphere microbiome.

Keywords Amplicon sequencing · Phenolics · *Ralstonia solanacearum* · Root exudation · Root-pathogen interaction · Soil microbiome

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Introduction

Plants invest a considerable fraction of their photosynthesized carbon into root exudates, a collection of low-molecular-weight compounds released into the rhizosphere (Bais et al. 2006). These exudates mediate complex interactions between plants and soil microbes and are essential in structuring the composition of soil microbiome (Carvalhais et al. 2015; Chaparro et al. 2013; Lagos et al. 2014). One key function of root exudates is to suppress pathogenic microorganisms (Bais et al. 2005), which is largely mediated by phenolic compounds (Badri et al. 2013; Lanoue et al. 2009). This function can be direct, for instance, by inhibiting the growth of pathogen (Ling et al. 2013). Alternatively, phenolic compounds could affect the pathogen indirectly, for instance, by modulating the expression of antibiotic-related genes of non-pathogenic soil microbes (de Werra et al. 2011). Such indirect effects could be very important, as the rhizosphere is enriched with mutualistic microbes that can protect plants against diseases (Li et al. 2015; Qiu et al. 2013; Trivedi et al. 2011) by producing antimicrobial compounds and lytic enzymes, stimulating plant immunity, and intensifying competition for resources with the pathogen (Berendsen et al. 2012; Yu et al. 2014).

Disruption of the pathogen response-related jasmonic acid pathway alters root exudation patterns and the composition of rhizosphere microbial communities (Carvalhais et al. 2015) in *Arabidopsis thaliana*, confirming that shifts in exudation may be an integral part of plant response to pathogens. In this study, we addressed whether challenging plants with a pathogen alters the composition of soil microbiome via shifts in root exudation profile.

Exudation is very dynamic and depends on the plant growth stage (Chaparro et al. 2013) and the presence of pathogen. For example, the presence of the pathogenic fungus *Fusarium graminearum* in the rhizosphere of barley triggers the exudation of many phenolic compounds that prevent spore germination (Lanoue et al. 2009). Similarly, alterations of phenolic compound exudation in barley infected with the oomycete *Pythium ultimum* induce expression of antibiotic-related genes in *Pseudomonas protegens* (Jousset et al. 2011).

In this study, we assessed whether challenging plants with a pathogen leads to shifts in exudation patterns. We further assessed whether pathogen-induced exudates could inhibit growth of the pathogen and alter microbiome composition. We challenged tomato plants with *Ralstonia solanacearum*, a cosmopolite pathogen which causes bacterial wilt in more than 200 host species (Salanoubat et al. 2002). In order to disentangle the plant-mediated effects from pathogen-induced disturbance, we collected tomato exudates in the absence and presence of *R. solanacearum*. We sterilized the exudates and added them to an unplanted soil to mimic rhizosphere condition without direct pathogen influence. We then compared the effects of the different exudates on soil microbiome composition and linked them to changes in exudate composition.

Materials and methods

Bacterial strain and plant species

The bacterial pathogen *R. solanacearum* strain QL-Rs1115 (Wei et al. 2011) was routinely cultivated in NB medium (10.0 g of glucose, 5.0 g of peptone, 0.5 g of yeast extract, and 3.0 g of beef extract in 1 L of H_2O at pH 7.0). Overnight-grown bacteria were harvested by centrifugation (10,000×*g* for 6 min), washed twice with sterile saline solution (0.9 % NaCl), and diluted to appropriate concentrations based on their optical density (OD_{600}).

Solanum lycopersicum cv. 'Micro-Tom' tomato was used as a model plant species. Seeds were surface sterilized with NaClO (3 % *v*/*v*) for 10 min and rinsed four times with sterile distilled water. Surface-sterilized seeds were then plated on Murashige and Skoog agar medium (Murashige and Skoog 1962) supplemented with 1 % sucrose and incubated in the dark at 28 °C for 2 days, until the emergence of roots.

Collection of root exudates during the pathogen challenge

Root exudates were collected based on a previously published methodology (Badri et al. 2009) with minor modifications. Briefly, 2-day-old tomato seedlings were transferred to six-well culture plates (Corning, CA, USA); each well contained two seedlings in 2 ml of liquid Murashige and Skoog medium amended with 1 % sucrose. Plates were incubated on an orbital shaker at 90 rpm and exposed to white fluorescent light (50 μmol m^{-2} s^{-1}) with a 16:8-h light/dark photoperiod at 25 ± 2 °C. After 10 days, plants were gently washed with sterile double-distilled water to remove the remaining exudates and transferred to new six-well culture plates containing 2 ml of sterile double-distilled water per well. Sterilized double-distilled water was used to prevent the medium from interfering with the subsequent high-performance liquid chromatography (HPLC) analyses (Badri et al. 2013). We set up three treatments: (a) *R. solanacearum* grown alone, (b) tomato plants grown alone, and (c) tomato plants grown with *R. solanacearum*. Plants were inoculated with 20 μl of a bacterial suspension (OD_{600} = 0.5; 2 × 10^8 CFU ml^{-1} in 0.9 % NaCl) or 20 μl of 0.9 % NaCl. To obtain secretion of *R. solanacearum*, sterilized double-distilled water was inoculated with 20 μl of a bacterial suspension (OD_{600} = 0.5; 2 × 10^8 CFU ml^{-1} in 0.9 % NaCl). Each treatment had three replicates, and each replicate contained pooled exudate from 12 wells (i.e., 24 plants). Liquid medium was collected 72 h after pathogen inoculation, and the pathogen survival was measured by serial dilution plating on NA medium (10.0 g of glucose, 5.0 g of peptone, 0.5 g of yeast extract, 3.0 g of beef extract, and 15 g of agar in 1 L of H_2O at pH 7.0). Pooled samples were centrifuged (10,000×*g* for 6 min) and sterile-filtered (0.22 μm) to remove the pathogen and root cells. Samples were then lyophilized and redissolved in 300 μl of solvent (methanol/water = 30:70; *v*/*v*). Similar to experiments conducted with *A. thaliana* (Badri et al. 2013; Rudrappa et al. 2008), the tomato plants incubated in sterile double-distilled water did not show any visible nutrient deficiency symptoms or toxicity symptoms during the 3-day sampling period. We collected exudates over a short time period corresponding to the latency phase of the infection, during which infection remained asymptomatic (Jacobs et al. 2012; Milling et al. 2011). This allowed us to measure the plant response while avoiding biases due to disease onset.

HPLC analyses

In this study, we focused on antimicrobial phenolics present in the tomato root exudates. An 20 μl aliquot of each sample was injected for HPLC analysis following a previously described method (Ling et al. 2010). Briefly, separation was performed by gradient elution using an Agilent 1200 system (Agilent Technologies, CA, USA) with an XDB-C18 column

(4.6 mm × 250 mm). The solvent system consisted of acetonitrile and 2 % (*v*/*v*) acetic acid. The UV-visible photodiode detector was set to 280-nm wavelength. Peaks were identified by comparing their retention times with standards that were run under the same conditions (Rudrappa et al. 2008). The standard phenolic compounds used in this study were caffeic acid, cinnamic acid, coumaric acid, syringic acid, ferulic acid, β-hydroxybenzoic acid, gallic acid, benzoic acid, phthalic acid, and vanillic acid.

Effects of root exudates on microbiome composition in plant-free soil microcosms

The soil used in this assay was collected from a tomato field in Qilin (118° 57′ E, 32° 03′ N), Nanjing, China, which shows a high bacterial wilt incidence. The soil is a yellow-brown earth (Udic Argosol) with the following properties: pH 5.4, organic matter (OM) content of 24.6 g kg^{-1}, total N of 6.3 g kg^{-1}, available P of 172.9 mg kg^{-1}, and available K of 178 mg kg^{-1}. The soil was first cleared of plant debris, sieved (<2 mm), homogenized thoroughly, and transferred to 24-well culture plates, with each well receiving 1.8 g of soil (dry weight). The exudates of 60 plants for each replicate were collected by the method described above and redissolved in 7.5 ml of solvent (methanol/water = 30:70; *v*/*v*) before being used to supplement the soil. We set up four different exudate treatments (250 μl day^{-}): (a) solvent only (methanol/water = 30:70; *v*/*v*; control), (b) exudates produced in the absence of a pathogen, (c) exudates produced in the presence of a pathogen, and (d) caffeic acid (3.6 mM; i.e., 0.5 μmol g^{-1} day^{-1}). Each treatment had three replicates, and each replicate well received 250 μl of exudate solution per day for a total of 30 days. The caffeic acid treatment was set up to assess its specific role in modulating the composition of soil bacterial community when tomato roots were challenged with *R. solanacearum*. The amount of caffeic acid entering the soil (i.e., 0.5 μmol g^{-1} day^{-1}) was based on previous studies (Eilers et al. 2010; Paterson et al. 2007; Qu and Wang 2008; Zhou and Wu 2012). The 24-well plates were weighed each day and were replenished with sterile distilled water to maintain the soil moisture at 60 % of its maximum water holding capacity. Plates were incubated in a growth chamber with a 16:8-h light/dark photoperiod at 25 ± 2 °C to mimic natural conditions. At the end of the soil microcosm experiment, all the soils were collected, thoroughly homogenized, and stored at −80 °C.

Total DNA extraction and 16S rRNA gene amplicon sequencing

To characterize changes in the soil microbiome composition, soil DNA was extracted from 300-mg soil using the PowerSoil DNA Isolation Kit (Mo Bio, Carlsbad, CA, USA) according to the manufacturer's instructions. Three DNA extracts of each replicate were pooled and quantified using a NanoDrop (Thermo Scientific, Wilmington, DE, USA). The V4 hypervariable regions of the bacterial 16S rRNA gene sequences analysis were PCR amplified using the primer pairs 563F (5′-AYTGGGYDTAAAGVG-3′) and 802R (5′-TACNVGGGTATCTAATCC-3′) (Cardenas et al. 2010) with the following PCR conditions: the reaction mix (20 μl) contained 4 μl of 5× Fast-Pfu buffer, 2 μl of 2.5 mM dNTPs, 0.4 μl of each primer (5 μM), 0.5 μl of DNA sample, and 0.4 μl of Fast-Pfu polymerase (TransGen Biotech, Beijing, China). PCR amplification included 30 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s in an Applied Biosystems thermal cycler (GeneAmp PCR system 9700, Applied Biosystems, Foster City, CA, USA). For each DNA sample, three independent PCRs were performed and the triplicate products were pooled to minimize the bias of PCR amplification. The amplicon products were purified using an AxyPrep PCR Clean-up Kit (Axygen Biosciences, Union City, CA, USA) before perfoming agarose gel electrophoresis. The concentrations of the purified PCR products were determined with QuantiFluor™-ST (Promega, WI, USA) before subjecting them to 250-nucleotide paired-end sequencing using an Illumina MiSeq platform at Shanghai Majorbio Bio-pharm Bio-technology Co., Ltd.

16S rRNA gene sequencing analysis

The sequence data were processed following the UPARSE pipeline (Edgar 2013). Briefly, read pairs from each sample were assembled, low-quality nucleotides (maximal expected error of 0.25) were removed, and reads shorter than 200 bp were discarded. After elimination of singletons, sequence reads were clustered into operational taxonomic units (OTUs) at a threshold of 97 % similarity, followed by removal of chimeras using the UCHIME method (Edgar et al. 2011). The representative sequences and OTU tables obtained using the UPARSE pipeline were then analyzed using Mothur (Schloss et al. 2009). Sampling depth was equalized to the depth of the smallest sample (31,200 reads). The taxonomies of each OTUs were annotated using the RDP 16S rRNA classifier (Wang et al. 2007) with a confidence threshold of 80 %. The composition of the bacterial community was clustered based on unweighted Unifrac distance metrics (Lozupone et al. 2007).

Influence of caffeic acid on the growth of *R. solanacearum*

Bacteria were grown in 96-well culture plates with each well containing 188 μl of diluted (1:5) NB medium, 2 μl of bacterial suspension (OD_{600} = 0.5), and 10 μl of caffeic acid (prepared in pure ethanol; Sigma-Aldrich, St. Louis, MO, USA) at a final concentration of 0, 5, 10, 20, 40, 80, 120, or 160 μM. Plates were incubated at 30 °C with shaking (170 rpm). Bacterial growth was determined by measuring the optical

density at 600 nm using a SpectraMax M5 (Molecular Devices, Sunnyvale, CA, USA). The percentage of growth inhibition was calculated according to the following formula: Percentage of growth inhibition = (Bacterial growth in the absence of caffeic acid − Bacterial growth in the presence of caffeic acid) × Bacterial growth in the absence of caffeic acid^{-1} × 100 %.

Statistical analysis and sequence accession number

Analysis of variance (ANOVA, Duncan's multiple range test) and Student's *t* test were used to compare mean differences between the treatments by using SPSS (v. 19). Redundancy analysis (RDA) was performed using CANOCO (ETTEN 2005). Effect of caffeic acid on the growth of *R. solanacearum* QL-Rs1115 was assessed with a linear model. All of the raw sequences have been deposited in the DDBJ SRA under the accession number SRP068343.

Results

The effect of *R. solanacearum* presence on tomato root exudate profile

Redundancy analysis (RDA) showed clearly different root exudate profiles in the three different treatments (Fig. 1a). The first two principal coordinates explained 88.9 % of the total variation of secretions among the individual samples. RDA and Monte Carlo permutation test (499 unrestricted permutations) were used to identify the HPLC peaks that significantly influenced the overall chromatographic profile. Our results revealed that compounds 2 ($F = 24.0$, $p = 0.004$), 4 ($F = 23.9$, $p = 0.002$), 10 ($F = 15.9$, $p = 0.006$), and 13 ($F = 21.2$, $p = 0.002$) were found at significantly higher concentrations in the tomato exudates compared to pathogen-only samples. In contrast, compounds 1 ($F = 22.3$, $p = 0.002$), 3 ($F = 22.3$, $p = 0.02$), 7 ($F = 22.3$, $p = 0.008$) and 12 ($F = 5.4$, $p = 0.016$) were more abundant in pathogen-only samples compared to plant-only or plant-and-pathogen samples. Crucially, pathogen presence changed the tomato root exudate profile by favoring the secretion of compounds 5 ($F = 4.6$, $p = 0.008$) and 15 ($F = 7.5$, $p = 0.008$). The compound 15 was further determined to be caffeic acid by comparing its retention time with known standards. Chromatographic profiles revealed a significant increase ($p = 0.002$, Student's *t* test) of caffeic acid under pathogen infection (Figs. 1b and S1). While caffeic acid was also detected from plant-only samples, it was never detected in pathogen-only samples, suggesting that it was produced by the plant and not the pathogen. Compound 5 was detected only in the plant-and-pathogen samples. Unfortunately, we were not able to identify all other compounds except caffeic acid. This could be due to the lack of suitable reference standards or potentially low compound solubility in the solvent (Carvalhais et al. 2015).

The effect of tomato exudates and caffeic acid on the soil microbiome composition

At the end of the experiment, soils inoculated with the solvent (control) had the highest bacterial OTU richness (Fig. 2a). Bacterial OTU richness of soil treated with *R. solanacearum*-infected plant root exudates was significantly ($p = 0.007$, Student's *t* test) lower than samples

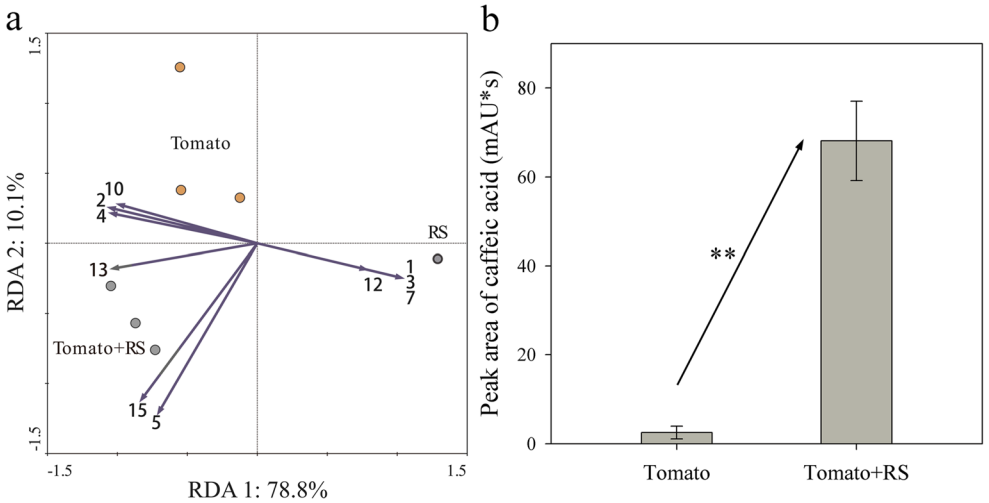

Fig. 1 Variation in the composition of exudates originating from pathogen-only (RS), plant-only (tomato), and plant-and-pathogen (tomato + RS) treatments as detected by HPLC. **a** The relationship between individual exudate compounds and root exudate composition as determined by RDA. The percentage of the explained variation is indicated on *X* and *Y* axes. Individual exudate compounds that were significantly ($p < 0.05$) correlated with the exudate composition are presented as *arrows*. *Numbers* indicate peaks on the HPLC chromatogram. **b** The difference in the caffeic acid exudation in the absence and presence of *R. solanacearum*. *Bars* show mean values ± SE ($n = 3$). **$p < 0.01$

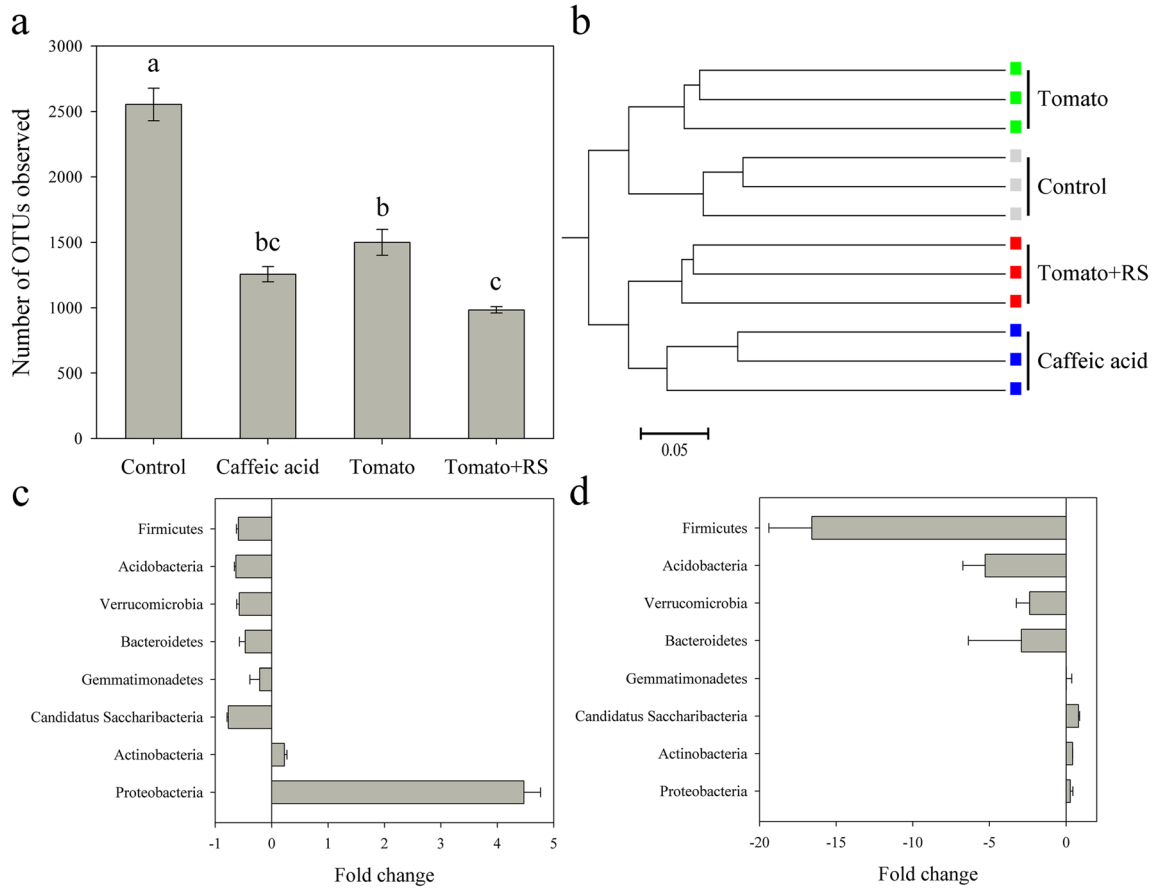

Fig. 2 The effect of plant exudates and caffeic acid on the soil microbiome composition. **a** Bacterial OTU richness in control, caffeic acid, plant-only (tomato), and plant-and-pathogen (tomato + RS) treatments. *Different letters* indicate significant differences. **b** Community similarity based on the cluster analysis of unweighted Unifrac metrics. **c** The fold change of bacterial phyla in the plant-and-pathogen exudate treatment relative to the plant-only exudate treatment. **d** The fold change of bacterial phyla in caffeic acid treatment relative to the control. *Bars* show mean values ± SE ($n = 3$)

treated with non-infected plant exudates. Cluster analyses based on the unweighted Unifrac metrics showed that bacterial community replicates from the same treatment clustered together (Fig. 2b). Non-infected plant exudate treatment clustered with the control (soil treated with 30 % methanol), and *R. solanacearum*-infected plant root exudate treatment clustered with the caffeic acid treatment, suggesting that addition of caffeic acid could mimic the effects of pathogen-induced shifts in root exudates. We further classified the soil bacterial communities into phylotypes consisting of eight major bacterial phyla (Fig. S2). In contrast to non-infected plant exudate treatment, *R. solanacearum*-infected plant exudate treatment was associated with an increase abundance of *Proteobacteria* and *Actinobacteria* and reduced abundance of *Firmicutes*, *Acidobacteria*, *Verrucomicrobia*, *Bacteroidetes*, *Gemmatimonadetes*, and *Candidatus Saccharibacteria* (Fig. 2c). Caffeic acid and infected plant exudates had fairly similar effects on microbial community composition. For example, caffeic acid treatment was also associated with an increased abundance of *Proteobacteria* and *Actinobacteria* and reduced abundance of *Firmicutes*, *Acidobacteria*, and *Verrucomicrobia* compared to control (Fig. 2d).

Correlation between bacterial community composition and root exudates

Changes in the abundance of 20 major bacterial genera (47.9 % by average relative abundance) were associated with changes in the concentration of specific exudates (Fig. 3). RDA ordination by vector fitting revealed compound 5 ($F = 13.4$, $p = 0.002$, Monte Carlo) and caffeic acid ($F = 12.5$, $p = 0.002$, Monte Carlo) as significantly correlated exudate components. For example, positive correlations were observed between caffeic acid; the unidentified compound 5; and the genera *Brachybacterium*, *Janibacter*, *Dyella*, *Rhodanobacter*, and *Intrasporangium*, and these bacterial genera showed higher abundances in the plant-and-pathogen exudate treatment. In contrast, negative correlations were observed

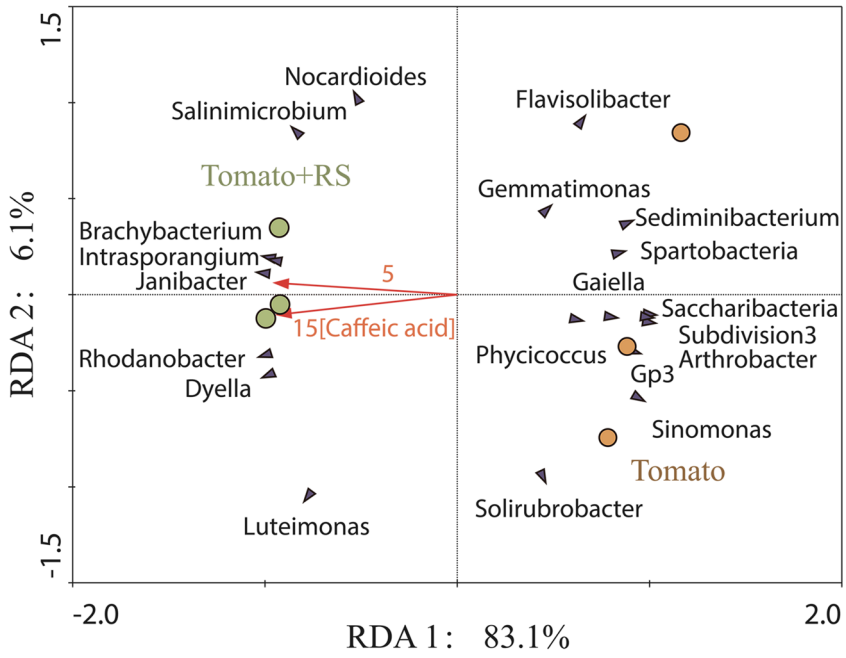

Fig. 3 RDA ordination summarizing the correlations between the top 20 genera of soil bacterial communities and selected exudate compounds in the plant-only (tomato) and plant-and-pathogen (tomato + RS) treatments (superimposed as fitted vectors). The *red arrows* show the magnitude (length) and correlation (angle) of individual exudate components that were significantly ($p < 0.05$) correlated with the ordination (Color figure online)

between caffeic acid or unidentified compound 5 and the genera *Saccharibacteria*, *Arthrobacter*, *Phycicoccus*, *Gaiella*, and *Subdivision 3*, and these bacterial genera showed higher abundance in the plant-only exudate treatment.

The effect of caffeic acid on *R. solanacearum* growth

To validate the role of caffeic acid in plant-pathogen interactions, the effects of pure caffeic acid on the growth of *R. solanacearum* QL-Rs1115 were measured in vitro. Caffeic acid moderately reduced the growth of *R. solanacearum* QL-Rs1115 in a dose-dependent way that could be well modeled with Michaelis-Menten kinetics ($R^2 = 0.66$, $p < 0.001$; Fig. 4). At concentrations above 80 μM, the effects of caffeic acid on the growth of *R. solanacearum* QL-Rs1115 reached plateau.

Discussion

Root exudates are crucial for modulating the interactions between plants and soil microbes (Bais et al. 2006). One of the main functions of these exudates is to directly suppress soil-borne pathogenic microorganisms (Bais et al. 2005). However, root exudates may also have indirect negative effects on the pathogens via changes in commensal rhizosphere microbiome composition. Here, we demonstrated shifts in root exudate profile and an elevated secretion of caffeic acid triggered by *R. solanacearum* invasion (Figs. 1 and S1). The shifts in root exudate profile further affect the composition of soil bacterial community (Figs. 2 and 3). We found that increased caffeic acid directly inhibited the growth of *R. solanacearum* QL-Rs1115 (Fig. 4). Together, these results suggest that pathogen invasion can activate plant defenses that inhibit pathogen growth directly and change the composition of soil microbiome indirectly via shifts in root exudation profile.

Correlation analyses have revealed close relationship between root exudate profiles and the activity, biomass, and composition of the rhizosphere microbiome (Badri et al. 2009; Haichar et al. 2008; Paterson et al. 2007). For example, an ATP-binding cassette transporter mutant of *Arabidopsis*, which increased the secretion of phenolics while reducing sugars

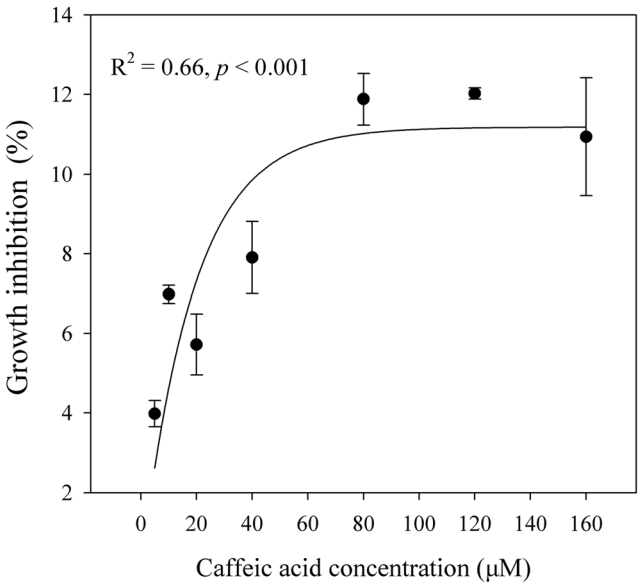

Fig. 4 The effect of caffeic acid on the growth of *R. solanacearum*. Growth of *R. solanacearum* QL-Rs1115 in 20 % NB media (after 24 h at 30 °C) was determined by measuring the absorbance at 600 nm (OD_{600}). The regression curve is based on Michaelis-Menten fitting. *Bars* show mean values ± SE ($n = 3$)

secretion, showed changes in the composition of rhizosphere bacterial and fungal communities compared to the wild type (Badri et al. 2009). Other studies have directly demonstrated that root exudates can considerably mediate the activity, biomass, and composition of soil microbiome through the application of artificial or natural blends of root secretions to soil samples in the absence of plants (Badri et al. 2013; Eilers et al. 2010; Paterson et al. 2007). In this study, we added exudates from healthy and *R. solanacearum*-infected tomato plants to a reference soil. Root exudates from healthy and *R. solanacearum*-infected tomato plants can help to assemble distinct soil microbiomes (Fig. 2b).

In contrast to non-infected plant exudate treatment, *R. solanacearum*-infected plant exudate treatment was associated with an increase abundance of *Proteobacteria* and *Actinobacteria* and reduced abundance of *Firmicutes*, *Acidobacteria*, *Verrucomicrobia*, *Bacteroidetes*, *Gemmatimonadetes*, and *Candidatus* Saccharibacteria (Fig. 2c). Crucially, the effects of infected plant exudates on the composition of soil bacterial community could be mimicked by adding pure caffeic acid to the soil (Fig. 2b): even though the effect of caffeic acid was not identical, it was also associated with an increased abundance of *Proteobacteria* and *Actinobacteria* and reduced abundance of *Firmicutes*, *Acidobacteria*, and *Verrucomicrobia* compared to the control (Fig. 2d). In addition, changes in the concentration of caffeic acid and unidentified compound 5 were also significantly (positively or negatively) correlated with changes in the abundances of several major bacterial genera (Fig. 3). These observations are in agreement with the results of Badri et al. (2013) where phenolic-related compounds were found to significantly (positively or negatively) correlate with a higher number of OTUs when compared with other classes of compounds such as sugars, amino acids, and sugar alcohols. These results thus suggest that caffeic acid may have played an important role in modulating the composition of soil bacterial community when tomato roots are challenged with *R. solanacearum* and phenolics in exudates may be the predominant modulators of soil bacterial community composition.

In this study, we show that the presence of different exudates can act as a filter and decrease the bacterial OTU richness of the soil bacterial community (Fig. 2a). Previous studies showed similar results that plant roots drive a reduction in the bacterial richness of the rhizosphere (Peiffer et al. 2013; Shi et al. 2015). In contrast to bulk soil, rhizospheric soil is generally considered to be enriched in fast-growing microbes, which respond positively to carbon sources (Fierer et al. 2007; Peiffer et al. 2013). Reductions in bacterial community diversity in our work may due to the selection or enrichment of specific fast-growing taxa. For example, *Proteobacteria* and *Actinobacteria*, which have generally been characterized as fast-growing phyla (Goldfarb et al. 2011), respond positively to caffeic acid addition compared to control (Fig. 2d). However, we still do not know whether reductions in OTU abundance correspond to reductions in bacterial functional diversity.

Accumulating evidence suggests that plant roots can secrete diverse protective metabolites upon pathogen infection, and phenolics in root exudates may function as general antimicrobial agents (Bais et al. 2004; 2005; Ling et al. 2010). For instance, barley, when challenged with the pathogen *F. graminearum*, rapidly induces the de novo synthesis of phenolic compounds that inhibit the germination of *F. graminearum* (Lanoue et al. 2009). Caffeic acid secreted by grafted watermelon is associated with resistance to *Fusarium oxysporum* (Ling et al. 2013). On the other hand, many phenolics in the root exudates can act as metal chelators and may change the availability of metallic soil micronutrients (Bais et al. 2006). For example, caffeic acid can chelate Cu(II) and alleviate its phytotoxicity (Garau et al. 2015), while the possibility remains that pathogenic bacteria use chelators as a strategy to acquire micronutrients essential for virulence and pathogenicity (Hood and Skaar 2012; Oide et al. 2006). Therefore, the complex impacts of root exudates and caffeic acid on the pathogen might have, in turn, multiple effects on plant health.

In this study, we only concentrated on bacterial interactions in this study. This excludes many other important soil microbial interactions with protist predators, phages, nematodes, and fungi (Berendsen et al. 2012). For example, mycorrhizal fungi have been shown to also affect the composition of rhizosphere bacterial community (Lioussanne et al. 2010), having effects on the functioning of the soil ecosystem (Van der Heijden et al. 1998; Vogelsang et al. 2006). As a result, we still need a better understanding of plant-pathogen interactions in more complex soil microbial communities. This information would be especially helpful from the applied perspective to guide how to manipulate the soil microbiome composition in order to improve the plant health and the crop yield (Chaparro et al. 2012; Xue et al. 2015). For example, in addition to adding artificial plant exudates, one could apply symbiotic mycorrhizal fungi into the soil to increase the suppression of pathogens (Borowicz 2001; Rodriguez and Sanders 2015).

In conclusion, here, we show that pathogen invasion causes clear changes in tomato root exudation profile by specifically increasing the secretion of phenolic compounds. This change had important effects on the composition of soil microbial community. The increased release of caffeic acid in root exudate had negative effect on the pathogen growth. Together, these results suggest that pathogen-plant interactions can have community-wide effects on the composition of soil microbial communities.

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