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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

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 antibiotics-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

68 germination (Lanoue et al. 2009). Similarly, alterations of phenolic compound exudation in barley
69 infected with the oomycete *Pythium ultimum* induce expression of antibiotics-related genes in
70 *Pseudomonas protegens* (Jousset et al. 2011).

71 In this study we assessed whether challenging plants with a pathogen leads to shifts in exudation
72 patterns. We further assessed whether pathogen-induced exudates could inhibit growth of the pathgen
73 and alter microbiome composition. We challenged tomato plants with *Ralstonia solanacearum,* a
74 cosmopolite pathogen which causes bacterial wilt in more than 200 host species (Salanoubat et al.
75 2002). In order to disentangle the plant-mediated effects from pathogen-induced disturbance, we
76 collected tomato exudates in the absence and presence of *R. solanacearum*. We sterilized the exudates
77 and added them to an unplanted soil to mimic rhizosphere condition without direct pathogen influence.
78 We then compared the effects of the different exudates on soil microbiome composition and linked
79 them to changes in exudate composition.

80 **Materials and methods**

81 **Bacterial strain and plant species**

82 The bacterial pathogen *R. solanacearum* strain QL-Rs1115 (Wei et al. 2011) was routinely cultivated in
83 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
84 of H_2O at pH 7.0). Overnight-grown bacteria were harvested by centrifugation (10,000×g for 6 min),
85 washed twice with sterile saline solution (0.9% NaCl) and diluted to appropriate concentrations based
86 on their optical density (OD_{600}).

87 *Solanum lycopersicum* cv. 'Micro-Tom' tomato was used as a model plant species. Seeds were
88 surface sterilized with NaClO (3% v:v) for 10 min and rinsed four times with sterile distilled water.
89 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 6-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 ten days, plants were gently washed with sterile double-distilled water to remove the remaining exudates and transferred to new 6-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 ager 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

112 (methanol:water = 30:70; v:v). Similar to experiments conducted with *A. thaliana* (Badri et al. 2013;
113 Rudrappa et al. 2008), the tomato plants incubated in sterile double-distilled water did not show any
114 visible nutrient deficiency symptoms or toxicity symptoms during the 3-day sampling period. We
115 collected exudates over a short time period corresponding to the latence phase of the infection, during
116 which infection remained asymptomatic (Jacobs et al. 2012; Milling et al. 2011). This allowed us to
117 measure the plant response while avoiding biases due to disease onset.

118 **HPLC analyses**

119 In this study, we focused on antimicrobial phenolics present in the tomato root exudates. An 20 µl
120 aliquot of each sample was injected for HPLC analysis following a previously described method (Ling
121 et al. 2010). Briefly, separation was performed by gradient elution using an Agilent 1200 system
122 (Agilent Technologies, CA, USA) with an XDB-C18 column (4.6 mm × 250 mm). The solvent system
123 consisted of acetonitrile and 2% (v:v) acetic acid. The UV-visible photodiode detector was set to 280
124 nm wavelength. Peaks were identified by comparing their retention times with standards that were run
125 under the same conditions (Rudrappa et al. 2008). The standard phenolic compounds used in this study
126 were caffeic acid, cinnamic acid, coumaric acid, syringic acid, ferulic acid, β-hydroxybenzoic acid,
127 gallic acid, benzoic acid, phthalic acid, and vanillic acid.

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

129 The soil used in this assay was collected from a tomato field in Qilin (118°57'E, 32°03'N), Nanjing,
130 China, which shows a high bacterial wilt incidence. The soil is a yellow-brown earth (Udic Argosol)
131 with following properties: pH 5.4, organic matter (OM) content of 24.6 g kg^{-1}, total N of 6.3 g kg^{-1},
132 available P of 172.9 mg kg^{-1}, and available K of 178 mg kg^{-1}. The soil was first cleared of plant debris,
133 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 d^{-1}): 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} d^{-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} d^{-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 amplicons 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 (ThermoScientific, Wilmington, DE, USA). The V4 hypervariable regions of the bacterial 16S rRNA gene were PCR-amplified using the primers pairs 563F (5'-AYTGGGYDTAAAGVG-3') and 802R (5'-TACNVGGGTATCTAATCC-3') (Cardenas et al. 2010) with the following PCR conditions: the reaction mix (20µl) contained 4 µl of 5X FastPfu 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 FastPfu 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-nuclotide paired-end sequencing using an Illumina MiSeq platform at Shanghai Majorbio Bio-pharm Bio-technology Co., Ltd.

16S rRNA 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*

178 Bacteria were grown in 96-well culture plates with each well containing 188 µl of diluted (1:5) NB
179 medium, 2 µl of bacterial suspension (OD_{600} = 0.5) and 10 µl of caffeic acid (prepared in pure ethanol;
180 Sigma-Aldrich, St. Louis, MO, USA) at a final concentration of 0, 5, 10, 20, 40, 80, 120 or 160 µM.
181 Plates were incubated at 30°C with shaking (170 rpm). Bacterial growth was determined by measuring
182 the optical density at 600 nm using a SpectraMax M5 (Molecular Devices, Sunnyvale, CA, USA). The
183 percentage of growth inhibition was calculated according to the following formula: Percentage of
184 growth inhibition = (Bacterial growth in the absence of caffeic acid - Bacterial growth in the presence
185 of caffeic acid) × Bacterial growth in the absence of caffeic acid $^{-1}$ × 100%.

186 **Statistical analysis and sequence accession number**

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

192 **Results**

193 **The effect of *Ralstonia solanacearum* presence on tomato root exudate profile**

194 Redundancy analysis (RDA) showed clearly different root exudate profiles in the three different
195 treatments (Fig. 1a). The first two principal coordinates explained 88.9% of the total variation of
196 secretions among the individual samples. Redundancy analysis and Monte Carlo permutation test (499
197 unrestricted permutations) were used to identify the HPLC peaks that significantly influenced the
198 overall chromatographic profile. Our results revealed that compounds 2 ($F = 24.0$, $p = 0.004$), 4 ($F =$
199 23.9, $p = 0.002$), 10 ($F = 15.9$, $p = 0.006$) and 13 ($F = 21.2$, $p = 0.002$) were found at significantly

200 higher concentrations in the tomato exudates compared to pathogen-only samples. In contrast,
201 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 =
202 0.016) were more abundant in pathogen-only samples compared to plant-only or plant-and-pathogen
203 samples. Crucially, pathogen presence changed the tomato root exudate profile by favoring the
204 secretion of compounds 5 (F = 4.6, p = 0.008) and 15 (F = 7.5, p = 0.008). The compound 15 was
205 further determined to be caffeic acid by comparing its retention time with known standards.
206 Chromatographic profiles revealed a significant increase (p = 0.002, Student's t test) of caffeic acid
207 under pathogen infection (Fig. 1b and S1). While caffeic acid was also detected from plant-only
208 samples, it was never detected in pathogen-only samples, suggesting that it was produced by the plant
209 and not the pathogen. Compound 5 was detected only in the plant-and-pathogen samples. Unfortunately,
210 we were not able to identify all other compounds except caffeic acid. This could be due to the lack of
211 suitable reference standards or potentially low compound solubility in the solvent (Carvalhais et al.
212 2015).

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

214 At the end of the experiment, soils inoculated with the solvent (control) had the highest bacterial OTU
215 richness (Fig. 2a). Bacterial OTU richness of soil treated with *R. solanacearum*-infected plant root
216 exudates was significantly (p = 0.007, Student's t test) lower than samples treated with non-infected
217 plant exudates. Cluster analyses based on the unweighted Unifrac metrics showed that bacterial
218 community replicates from the same treatment clustered together (Fig. 2b). Non-infected plant exudate
219 treatment clustered with the control (soil treated with 30% methanol) and *R. solanacearum*-infected
220 plant root exudate treatment clustered with the caffeic acid treatment suggesting that addition of caffeic
221 acid could mimic the effects of pathogen-induced shifts in root exudates. We further classified the soil

222 bacterial communities into phylotypes consisting of eight major bacterial phyla (Fig. S2). In contrast to
223 non-infected plant exudate treatment, *R. solanacearum*-infected plant exudate treatment was associated
224 with an increase abundance of *Proteobacteria* and *Actinobacteria* and reduced abundance of
225 *Firmicutes*, *Acidobacteria*, *Verrucomicrobia*, *Bacteroidetes*, *Gemmatimonadetes*, and *Candidatus*
226 Saccharibacteria (Fig. 2c). Caffeic acid and infected plant exudates had fairly similar effects on
227 microbial community composition. For example, caffeic acid treatment was also associated with an
228 increased abundance of *Proteobacteria* and *Actinobacteria* and reduced abundance of *Firmicutes*,
229 *Acidobacteria*, and *Verrucomicrobia* compared to control (Fig. 2d).

230 **Correlation between bacterial community composition and root exudates**

231 Changes in the abundance of 20 major bacterial genera (47.9% by average relative abundance) were
232 associated with changes in the concentration of specific exudates (Fig. 3). RDA ordination by vector
233 fitting revealed that compound 5 ($F = 13.4$, $p = 0.002$, Mont Carlo) and caffeic acid ($F = 12.5$, $p = 0.002$,
234 Mont Carlo) as significantly correlated exudate components. For example, positive correlations were
235 observed between caffeic acid, the unidentified compound 5 and the genera *Brachybacterium*,
236 *Janibacter*, *Dyella*, *Rhodanobacter*, and *Intrasporangium*, and these bacterial genera showed higher
237 abundances in the plant-and-pathogen exudate treatment. In contrast, negative correlations were
238 observed between caffeic acid or unidentified compound 5 and the genera *Saccharibacteria*,
239 *Arthrobacter*, *Phycicoccus*, *Gaiella*, and *Subdivision 3*, and these bacterial genera showed higher
240 abundance in the plant-only exudate treatment.

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

242 To validate the role of caffeic acid in plant-pathogen interactions, the effects of pure caffeic acid on the
243 growth of *R. solanacearum* QL-Rs1115 were measured *in vitro*. Caffeic acid moderately reduced the

244 growth of *R. solanacearum* QL-Rs1115 in a dose-dependent way that could be well modeled with
245 Michaelis-Menten kinetics ($R^2 = 0.66$, $p < 0.001$; Fig. 4). At concentrations above 80 μM, the effects of
246 caffeic acid on the growth of *R. solanacearum* QL-Rs1115 reached plateau.

247 **Discussion**

248 Root exudates are crucial for modulating the interactions between plants and soil microbes (Bais et al.
249 2006). One of the main functions of these exudates is to directly suppress soil-borne pathogenic
250 microorganisms (Bais et al. 2005). However, root exudates may also have indirect negative effects on
251 the pathogens via changes in commensal rhizosphere microbiome composition. Here we demonstrated
252 shifts in root exudate profile and an elevated secretion of caffeic acid triggered by *R. solanacearum*
253 invasion (Fig. 1 and S1). The shifts in root exudate profile further affect the composition of soil
254 bacterial community (Fig. 2 and 3). We found that increased caffeic acid directly inhibited the growth
255 of *R. solanacearum* QL-Rs1115 (Fig. 4). Together these results suggest that pathogen invasion can
256 activate plant defences that inhibit pathogen growth directly and change the composition of soil
257 microbiome indirectly via shifts in root exudation profile

258 Correlation analyses have revealed close relationship between root exudate profiles and the
259 activity, biomass and composition of the rhizosphere microbiome (Badri et al. 2009; Haichar et al.
260 2008; Paterson et al. 2007). For example, an ATP-binding cassette transporter mutant of *Arabidopsis*,
261 which increased the secretion of phenolics while reducing sugars secretion, showed changes in the
262 composition of rhizosphere bacterial and fungal communities compared to the wild type (Badri et al.
263 2009). Other studies have directly demonstrated that root exudates can considerably mediate the
264 activity, biomass and composition of soil microbiome through the application of artificial or natural
265 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 *Ralstonia solanacearum*-infected tomato plants to a reference soil. Root exudates from healthy and *Ralstonia 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;

288 Shi et al. 2015). In contrast to bulk soil, rhizospheric soil is generally considered to be enriched in
289 fast-growing microbes which respond positively to carbon sources (Fierer et al. 2007; Peiffer et al.
290 2013). Reductions in bacterial community diversity in our work may due to the selection or enrichment
291 of specific fast-growing taxa. For example, *Proteobacteria* and *Actinobacteria*, which have generally
292 been characterized as fast-growing phyla (Goldfarb et al. 2011), respond positively to caffeic acid
293 addition compared to control (Fig. 2d). However, we still do not know whether reductions in OTU
294 abundance correspond to reductions in bacterial functional diversity.

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

307 In this study, we only concentrated on bacterial interactions in this study. This excludes many
308 other important soil microbial interactions with protist predators, phages, nematodes and fungi
309 (Berendsen et al. 2012). For example, mycorrhizal fungi have been shown to also affect the

310 composition of rhizosphere bacterial community (Lioussanne et al. 2010), having effects on the
311 functioning of the soil ecosystem (Van der Heijden et al. 1998; Vogelsang et al. 2006). As a result, we
312 still need a better understanding of plant-pathogen interactions in more complex soil microbial
313 communities. This information would be especially helpful from the applied perspective to guide how
314 to manipulate the soil microbiome composition in order to improve the plant health and the crop yield
315 (Chaparro et al. 2012; Xue et al. 2015). For example, in addition to adding artificial plant exudates, one
316 could apply symbiotic mycorrhizal fungi into the soil to increase the suppression of pathogens
317 (Borowicz 2001; Rodriguez and Sanders 2015).

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

323

324 **References**

325 Badri DV, Chaparro JM, Zhang R, Shen Q, Vivanco JM (2013) Application of Natural Blends of
326 Phytochemicals Derived from the Root Exudates of Arabidopsis to the Soil Reveal That
327 Phenolic-related Compounds Predominantly Modulate the Soil Microbiome. J Biol Chem
328 288:4502-4512. doi:10.1074/jbc.M112.433300

329 Badri DV, Quintana N, El Kassis EG, Kim HK, Choi YH, Sugiyama A, Verpoorte R, Martinoia E,
330 Manter DK, Vivanco JM (2009) An ABC transporter mutation alters root exudation of
331 phytochemicals that provoke an overhaul of natural soil microbiota. Plant Physiol

151:2006-2017. doi:10.1104/pp.109.147462

Bais HP, Park S-W, Weir TL, Callaway RM, Vivanco JM (2004) How plants communicate using the underground information superhighway. Trends Plant Sci 9:26-32. doi:10.1016/j.tplants.2003.11.008

Bais HP, Prithiviraj B, Jha AK, Ausubel FM, Vivanco JM (2005) Mediation of pathogen resistance by exudation of antimicrobials from roots. Nature 434:217-221. doi:10.1038/nature09809

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233-266. doi:10.1146/annurev.arplant.57.032905.105159

Berendsen RL, Pieterse CM, Bakker PA (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478-486. doi:10.1016/j.tplants.2012.04.001

Borowicz VA (2001) Do arbuscular mycorrhizal fungi alter plant-pathogen relations? Ecology 82:3057-3068. doi:10.1890/0012-9658(2001)082[3057:DAMFAP]2.0.CO;2

Cardenas E, Wu WM, Leigh MB, Carley J, Carroll S, Gentry T, Luo J, Watson D, Gu B, Ginder-Vogel M, Kitanidis PK, Jardine PM, Zhou J, Criddle CS, Marsh TL, Tiedje JM (2010) Significant association between sulfate-reducing bacteria and uranium-reducing microbial communities as revealed by a combined massively parallel sequencing-indicator species approach. Appl Environ Microbiol 76:6778-6786. doi:10.1128/AEM.01097-10

Carvalhais LC, Dennis PG, Badri DV, Kidd BN, Vivanco JM, Schenk PM (2015) Linking Jasmonic Acid Signaling, Root Exudates, and Rhizosphere Microbiomes. Mol Plant-Microbe Interact 28:1049-1058. doi:10.1094/MPMI-01-15-0016-R

Chaparro JM, Badri DV, Bakker MG, Sugiyama A, Manter DK, Vivanco JM (2013) Root exudation of

354 phytochemicals in Arabidopsis follows specific patterns that are developmentally programmed
355 and correlate with soil microbial functions. PLoS One 8:e55731.
356 doi:10.1371/journal.pone.0055731
357 Chaparro JM, Sheflin AM, Manter DK, Vivanco JM (2012) Manipulating the soil microbiome to
358 increase soil health and plant fertility. Biol Fertil Soils 48:489-499.
359 doi:10.1007/s00374-012-0691-4
360 de Werra P, Huser A, Tabacchi R, Keel C, Maurhofer M (2011) Plant- and microbe-derived compounds
361 affect the expression of genes encoding antifungal compounds in a pseudomonad with
362 biocontrol activity. Appl Environ Microbiol 77:2807-2812. doi:10.1128/AEM.01760-10
363 Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat
364 Methods 10:996-998. doi:10.1038/nmeth.2604
365 Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and
366 speed of chimera detection. Bioinformatics 27:2194-2200. doi:10.1093/bioinformatics/btr381
367 Eilers KG, Lauber CL, Knight R, Fierer N (2010) Shifts in bacterial community structure associated
368 with inputs of low molecular weight carbon compounds to soil. Soil Biol Biochem 42:896-903.
369 doi:10.1016/j.soilbio.2010.02.003
370 ETTEN EV (2005) Multivariate analysis of ecological data using CANOCO. Austral Ecol 30:486-487.
371 doi:10.1111/j.1442-9993.2005.01433.x
372 Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria.
373 Ecology 88:1354-1364. doi:10.1890/05-1839
374 Garau G, Mele E, Castaldi P, Lauro GP, Deiana S (2015) Role of polygalacturonic acid and the
375 cooperative effect of caffeic and malic acids on the toxicity of Cu(II) towards triticale plants

376 (× Triticosecale Wittm). Biol Fertil Soils 51:535-544. doi:10.1007/s00374-015-0999-y
377 Goldfarb KC, Karaoz U, Hanson CA, Santee CA, Bradford MA, Treseder KK, Wallenstein MD, Brodie
378 EL (2011) Differential growth responses of soil bacterial taxa to carbon substrates of varying
379 chemical recalcitrance. Front Microbiol 2:94. doi:10.3389/fmicb.2011.00094
380 Haichar FZ, Marol C, Berge O, Rangel-Castro JI, Prosser JI, Balesdent J, Heulin T, Achouak W (2008)
381 Plant host habitat and root exudates shape soil bacterial community structure. ISME J
382 2:1221-1230. doi:10.1038/ismej.2008.80
383 Hood MI, Skaar EP (2012) Nutritional immunity: transition metals at the pathogen-host interface. Nat
384 Rev Microbiol 10:525-537. doi:10.1038/nrmicro2836
385 Jacobs JM, Babujee L, Meng F, Milling A, Allen C (2012) The in planta transcriptome of Ralstonia
386 solanacearum: conserved physiological and virulence strategies during bacterial wilt of tomato.
387 mBio 3:e00114-00112. doi:10.1128/mBio.00114-12
388 Jousset A, Rochat L, Lanoue A, Bonkowski M, Keel C, Scheu S (2011) Plants respond to pathogen
389 infection by enhancing the antifungal gene expression of root-associated bacteria. Mol
390 Plant-Microbe Interact 24:352-358. doi:10.1094/MPMI-09-10-0208
391 Lagos LM, Navarrete OU, Maruyama F, Crowley DE, Cid FP, Mora ML, Jorquera MA (2014)
392 Bacterial community structures in rhizosphere microsites of ryegrass (Lolium perenne var.
393 Nui) as revealed by pyrosequencing. Biol Fertil Soils 50:1253-1266.
394 doi:10.1007/s00374-014-0939-2
395 Lanoue A, Burlat V, Henkes GJ, Koch I, Schurr U, Röse US (2009) De novo biosynthesis of defense
396 root exudates in response to Fusarium attack in barley. New Phytol 185:577-588.
397 doi:10.1111/j.1469-8137.2009.03066.x

398 Li X, Zhang Yn, Ding C, Jia Z, He Z, Zhang T, Wang X (2015) Declined soil suppressiveness to
399 Fusarium oxysporum by rhizosphere microflora of cotton in soil sickness. Biol Fertil Soils
400 51:935-946. doi:10.1007/s00374-015-1038-8
401 Ling N, Huang Q, Guo S, Shen Q (2010) Paenibacillus polymyxa SQR-21 systemically affects root
402 exudates of watermelon to decrease the conidial germination of Fusarium oxysporum f.sp.
403 niveum. Plant Soil 341:485-493. doi:10.1007/s11104-010-0660-3
404 Ling N, Zhang W, Wang D, Mao J, Huang Q, Guo S, Shen Q (2013) Root exudates from grafted-root
405 watermelon showed a certain contribution in inhibiting Fusarium oxysporum f. sp. niveum.
406 PLoS One 8:e63383. doi:10.1371/journal.pone.0063383
407 Lioussanne L, Perreault F, Jolicoeur M, St-Arnaud M (2010) The bacterial community of tomato
408 rhizosphere is modified by inoculation with arbuscular mycorrhizal fungi but unaffected by
409 soil enrichment with mycorrhizal root exudates or inoculation with Phytophthora nicotianae.
410 Soil Biol Biochem 42:473-483. doi:10.1016/j.soilbio.2009.11.034
411 Lozupone CA, Hamady M, Kelley ST, Knight R (2007) Quantitative and Qualitative Diversity
412 Measures Lead to Different Insights into Factors That Structure Microbial Communities. Appl
413 Environ Microbiol 73:1576-1585. doi:10.1128/aem.01996-06
414 Milling A, Babujee L, Allen C (2011) Ralstonia solanacearum extracellular polysaccharide is a specific
415 elicitor of defense responses in wilt-resistant tomato plants. PLoS One 6:e15853.
416 doi:10.1371/journal.pone.0015853
417 Murashige T, Skoog F (1962) A Revised Medium for Rapid Growth and Bio Assays with Tobacco
418 Tissue Cultures. Physiol Plant 15:473-497. doi:10.1111/j.1399-3054.1962.tb08052.x
419 Oide S, Moeder W, Krasnoff S, Gibson D, Haas H, Yoshioka K, Turgeon BG (2006) NPS6, encoding a

nonribosomal peptide synthetase involved in siderophore-mediated iron metabolism, is a conserved virulence determinant of plant pathogenic ascomycetes. Plant Cell 18:2836-2853. doi:10.1105/tpc.106.045633

Paterson E, Gebbing T, Abel C, Sim A, Telfer G (2007) Rhizodeposition shapes rhizosphere microbial community structure in organic soil. New Phytol 173:600-610. doi:10.1111/j.1469-8137.2006.01931.x

Peiffer JA, Spor A, Koren O, Jin Z, Tringe SG, Dangl JL, Buckler ES, Ley RE (2013) Diversity and heritability of the maize rhizosphere microbiome under field conditions. Proc Natl Acad Sci U S A 110:6548-6553. doi:10.1073/pnas.1302837110

Qiu M, Li S, Zhou X, Cui X, Vivanco JM, Zhang N, Shen Q, Zhang R (2013) De-coupling of root–microbiome associations followed by antagonist inoculation improves rhizosphere soil suppressiveness. Biol Fertil Soils 50:217-224. doi:10.1007/s00374-013-0835-1

Qu XH, Wang JG (2008) Effect of amendments with different phenolic acids on soil microbial biomass, activity, and community diversity. Appl Soil Ecol 39:172-179. doi:10.1016/j.apsoil.2007.12.007

Rodriguez A, Sanders IR (2015) The role of community and population ecology in applying mycorrhizal fungi for improved food security. ISME J 9:1053-1061. doi:10.1038/ismej.2014.207

Rudrappa T, Czymmek KJ, Pare PW, Bais HP (2008) Root-secreted malic acid recruits beneficial soil bacteria. Plant Physiol 148:1547-1556. doi:10.1104/pp.108.127613

Salanoubat M, Genin S, Artiguenave F, Gouzy J, Mangenot S, Arlat M, Billault A, Brottier P, Camus J, Cattolico L (2002) Genome sequence of the plant pathogen Ralstonia solanacearum. Nature

415:497-502. doi:10.1038/415497a

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537-7541. doi:10.1128/AEM.01541-09

Shi S, Nuccio E, Herman DJ, Rijkers R, Estera K, Li J, da Rocha UN, He Z, Pett-Ridge J, Brodie EL, Zhou J, Firestone M (2015) Successional Trajectories of Rhizosphere Bacterial Communities over Consecutive Seasons. mBio 6:e00746. doi:10.1128/mBio.00746-15

Trivedi P, He Z, Van Nostrand JD, Albrigo G, Zhou J, Wang N (2011) Huanglongbing alters the structure and functional diversity of microbial communities associated with citrus rhizosphere. ISME J 6:363-383. doi:10.1038/ismej.2011.100

Van der Heijden MG, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69-72. doi:10.1038/23932

Vogelsang KM, Reynolds HL, Bever JD (2006) Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. New Phytol 172:554-562. doi:10.1111/j.1469-8137.2006.01854.x

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73:5261-5267. doi:10.1128/AEM.00062-07

Wei Z, Yang X, Yin S, Shen Q, Ran W, Xu Y (2011) Efficacy of Bacillus-fortified organic fertiliser in controlling bacterial wilt of tomato in the field. Appl Soil Ecol 48:152-159.

doi:10.1016/j.apsoil.2011.03.013

Xue C, Penton CR, Shen Z, Zhang R, Huang Q, Li R, Ruan Y, Shen Q (2015) Manipulating the banana rhizosphere microbiome for biological control of Panama disease. Sci Rep 5:11124. doi:10.1038/srep11124

Yu Z, Zhang Y, Luo W, Wang Y (2014) Root colonization and effect of biocontrol fungus Paecilomyces lilacinus on composition of ammonia-oxidizing bacteria, ammonia-oxidizing archaea and fungal populations of tomato rhizosphere. Biol Fertil Soils 51:343-351. doi:10.1007/s00374-014-0983-y

Zhou X, Wu F (2012) P-coumaric acid influenced cucumber rhizosphere soil microbial communities and the growth of Fusarium oxysporum f. Sp. Cucumerinum owen. PLoS One 7:e48288. doi:10.1371/journal.pone.0048288

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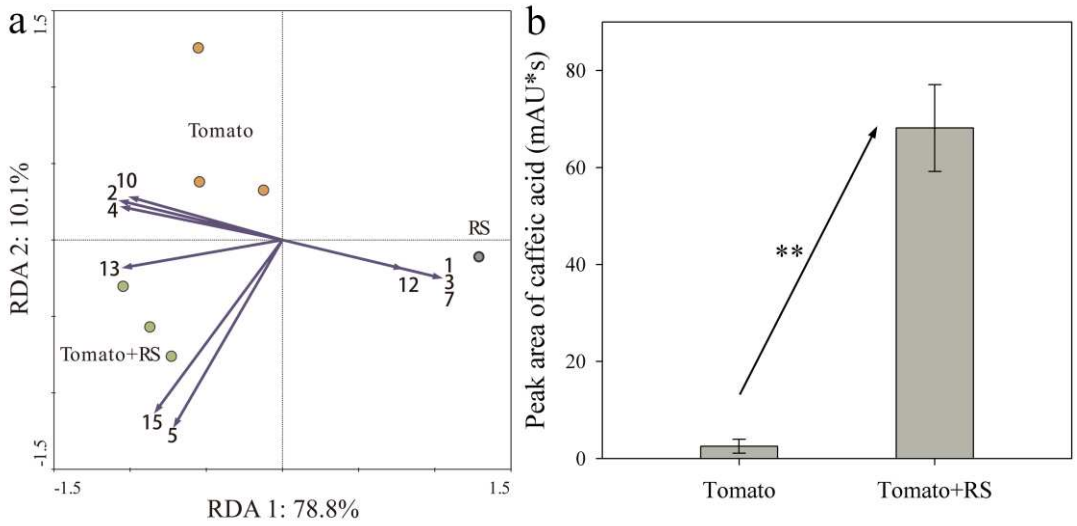

477

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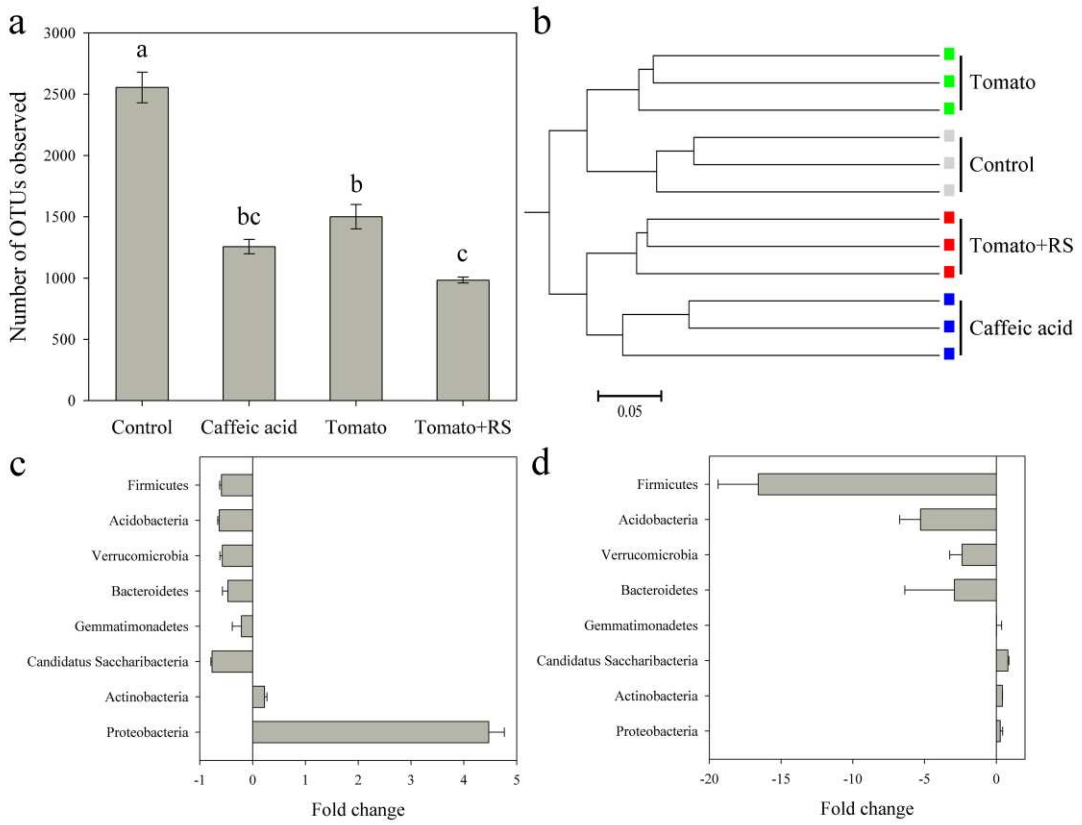
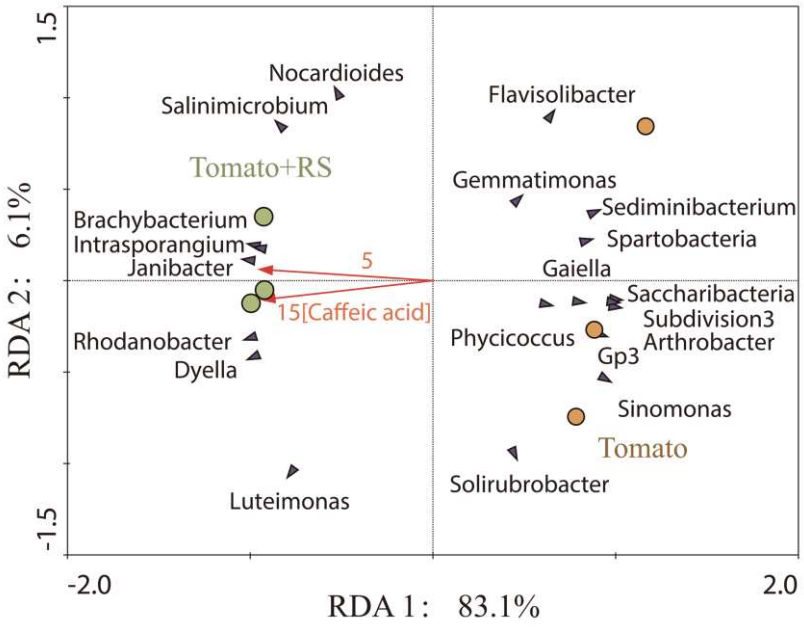


Fig. 2 The effect of plant exudates and caffeic acid on the soil microbiome composition. (a) Bacterial OTU richness in control, caffeic acid, plant-only (Tomato) and plant-and-pathogen (Tomato + RS) treatments. Different letters indicate significant differences. (b) Community similarity based on the 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)



494

495 **Fig. 3** RDA ordination summarizing the correlations between the top 20 genus of soil bacterial

496 communities and selected exudate compounds in the plant-only (Tomato) and plant-and-pathogen

497 (Tomato + RS) treatments (superimposed as fitted vectors). The red arrows show the magnitude (length)

498 and correlation (angle) of individual exudate components that were significantly ($p < 0.05$) correlated

499 with the ordination

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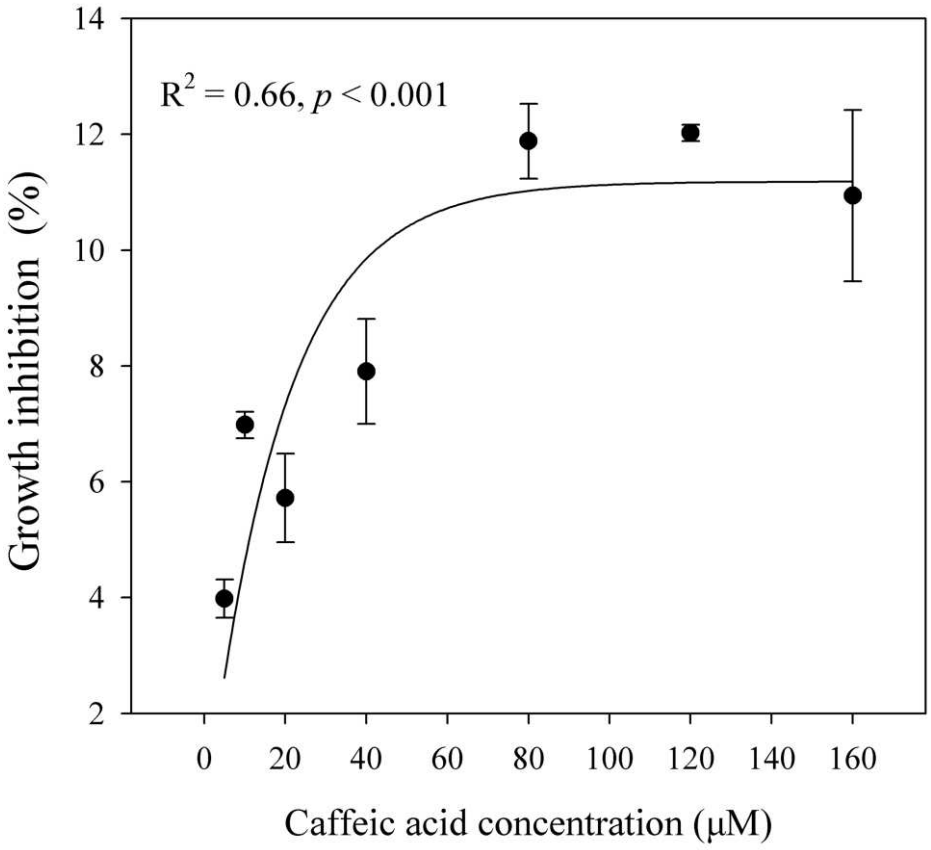

501

502 **Fig. 4** The effect of caffeic acid on the growth of *R. solanacearum*. Growth of *R. solanacearum*

503 QL-Rs1115 in 20% NB media (after 24 h at 30°C) was determined by measuring the absorbance at 600

504 nm (OD_{600}). The regression curve is based on Michaelis-Menten fitting. Bars show mean values ± SE

505 (n = 3)

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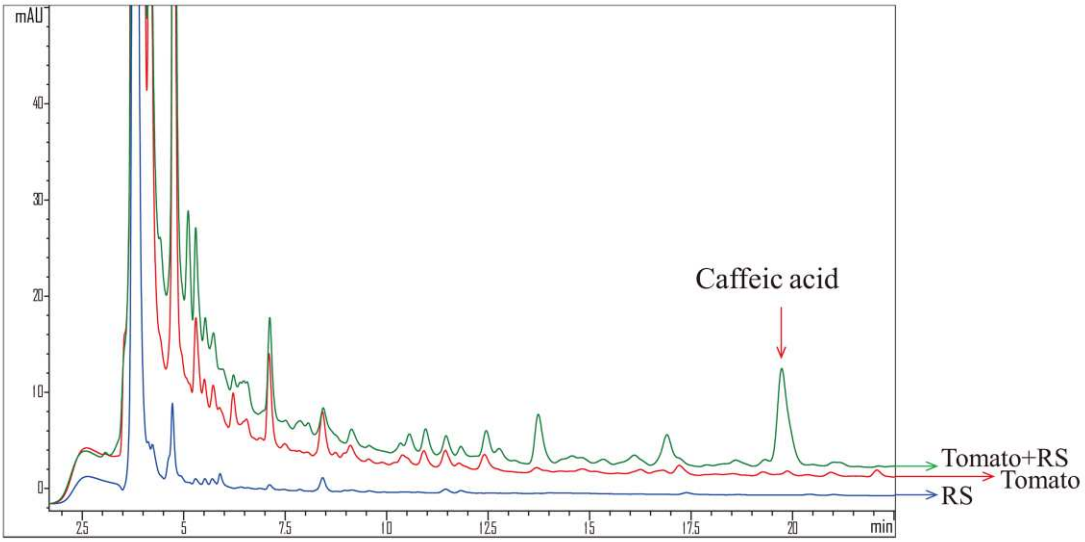

507

508 **Fig. S1** High-performance liquid chromatography (HPLC) profile of exudates originating from

509 pathogen-only (RS; blue line), plant-only (Tomato; red line) and plant-and-pathogen together (Tomato

510 + RS; green line) treatments

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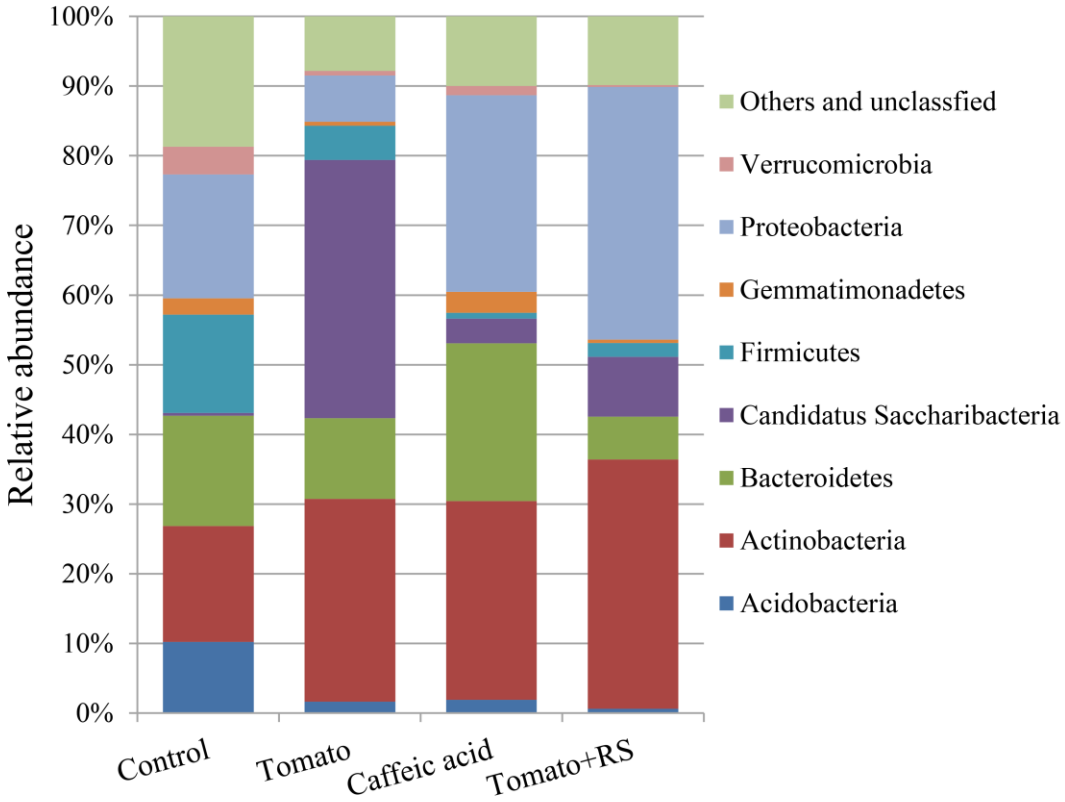

512

513 **Fig. S2** The relative abundance of the major bacterial phyla in the control, caffeic acid, plant-only

514 (Tomato) and plant-and-pathogen (Tomato + RS) treatments

515