Accepted Manuscript

Title: Activation of the salicylic acid signalling pathway in wheat had no significant short-term impact on the diversity of root-associated microbiomes

Authors: Hongwei Liu, Lilia C. Carvalhais, Peer M. Schenk, Paul G. Dennis

PII: S0031-4056(18)30091-X
DOI: https://doi.org/10.1016/j.pedobi.2018.08.001
Reference: PEDOBI 50541

To appear in:

Received date: 9-5-2018
Revised date: 2-8-2018
Accepted date: 6-8-2018

Please cite this article as: Liu H, Carvalhais LC, Schenk PM, Dennis PG, Activation of the salicylic acid signalling pathway in wheat had no significant short-term impact on the diversity of root-associated microbiomes, *Pedobiologia - Journal of Soil Ecology* (2018), https://doi.org/10.1016/j.pedobi.2018.08.001

Short communication:

Activation of the salicylic acid signalling pathway in wheat had no significant short-term impact on the diversity of root-associated microbiomes

Hongwei Liu[1], Lilia C. Carvalhais[2], Peer M. Schenk[1], Paul G. Dennis[3*]

[1]*School of Agriculture and Food Sciences, The University of Queensland, Brisbane, Queensland 4072, Australia*;

[2]*Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Ecosciences Precinct, GPO Box 267, Queensland 4001, Australia;*

[3]*School of Earth and Environmental Sciences, The University of Queensland, Brisbane, Queensland 4072, Australia*

*Correspondence: p.dennis@uq.edu.au

Highlights

- The salicylic acid (SA) pathway was activated in wheat grown in two soils.
- Root microbiomes were characterised for control and SA treated plants.
- SA treatment did not significantly affect the diversity of root microbiomes within 72 h.
- SA treatment is unlikely to help engineer beneficial wheat root microbiomes.

Abstract

Salicylic acid (SA) plays an important role in plant defence against biotrophic pathogens. Recent work with *Arabidopsis thaliana* mutants indicates an association between SA signalling and the diversity of root-associated microbial communities. This has led to the idea that activation of the SA pathway may help plants to rapidly recruit microbes that enhance stress tolerance and could be exploited as an approach to engineer beneficial plant microbiomes in agriculture. Nonetheless, unlike plants in natural environments, mutants with altered SA signalling constitutively express their phenotype. For this reason, we investigated whether transient activation of the SA pathway in wheat (*Triticum aestivum*) leads to rapid changes in the composition of root microbiomes. High throughput phylogenetic marker gene sequencing demonstrated that, 72 hours post-treatment, SA had no significant effects on the richness, evenness and composition of bulk soil and root-associated microbiomes in two soil types. These findings indicate that the structure of wheat root-associated microbiomes did not undergo significant rapid changes in response to activation of the SA signalling pathway.

Keywords: Plant defence; rhizosphere; endophytes; phytohormones; wheat; systemic acquired resistance

1. Introduction

Primary production is influenced by a wide-range of abiotic and biotic factors. With respect to the latter, microorganisms form intimate associations with plants that range from beneficial to deleterious. To protect themselves against attack, plants have evolved a wide-range of defences including the jasmonic acid (JA) pathway, which mediates defence against necrotrophic pathogens and herbivorous insects; and the salicylic acid (SA) pathway, which plays a crucial role in conferring local and systemic immunity against biotrophic and hemi-biotrophic pathogens (Glazebrook, 2005; Pieterse et al., 2009).

In *Arabidopsis thaliana*, the composition of root-associated microbial communities has been shown to respond to changes in SA (using mutants) (Lebeis et al., 2015) and JA signalling (using mutants and via exogenous application methyl jasmonate) (Carvalhais et al., 2013, 2015). Consequently, it has been suggested that these pathways help plants to recruit organisms that enhance host biotic stress tolerance, and could be exploited for agricultural applications (Lebeis et al*.,* 2015; Carvalhais et al., 2017). Exogenous hormonally-induced changes in root microbiomes have been shown to occur within just 72 h (Carvalhais et al., 2013; Liu et al., 2017a), and may be associated with shifts in root exudation (Carvalhais et al., 2015), which have been observed within the same timeframe (Badri et al., 2008). At present, however, the extent to which these pathways influence the microbiomes of commercially relevant crops is not clear. In wheat (*Triticum aestivum*), JA signalling has been shown to rapidly alter the composition of bacterial communities in endophytic root compartments, but not in the rhizosphere (Liu et al., 2017a). The effects of SA signalling on crop root microbiomes, however, have not been characterised.

Root exudates and other pools of rhizodeposits are known to strongly influence the composition and diversity of rhizosphere microbial communities (Carvalhais et al., 2015). SA treatment has been show to alter secondary metabolite profiles in a range of plant species (Badri and Vivanco 2009), and in *Arabidospis*, changes in root exudation have been observed as soon as 3-6 h post treatment (Badri et al., 2008). It is possible that these SA-induced changes in exudation help to attract microorganisms that enhance plant defences to biotrophic and hemi-biotrophic pathogens. Nevertheless, the involvement of soil microbes in assisting plant defence is typically associated with JA signalling through priming (Pieterse et al., 2009). For SA, effects of transient signalling on rhizosphere microbial community composition remain unknown. In this study, we tested the hypothesis that activation of the SA signalling pathway leads to rapid changes in the diversity of bacterial and archaeal communities associated with the roots of soil-grown wheat plants. To do this, plants were grown in pots under controlled conditions in two distinct soil types from the Northern Grains Region of Australia with a long history of wheat cultivation under no-till management. Half of the plants were sprayed with SA in 5% ethanol to exogenously activate the SA pathway, while the other half (the controls) were sprayed with 5% ethanol only. This approach has been used successfully in a range of plant species (Doornbos et al., 2011; Sardesai et al., 2005; Schenk et al., 2000). The diversity of root-associated microbial communities, 72-h post SA treatment, was then characterised using high throughput phylogenetic marker gene sequencing.

2. Materials and Methods

2.1 Plant growth conditions and experimental design

Wheat seeds (Crusader variety) were pre-germinated on moist filter paper for 36 h and then planted in 30-well punnet trays with three seeds per well, each containing c. 102 g soil (Fig. S1). A total of 360 plants were grown in a Calcisol (27.79°S, 150.20°E), and another 180 plants were grown in a Solonetz (26.90°S, 149.64°E) (IUSS Working Group WRB, 2007). These plants were allocated to two punnet trays as two treatments (control and SA-treated). Each treatment had three biological

replicates each comprising 30 plants (3 plants in each well) that were grown for 10 days post-planting. For the Calcisol, samples were collected 48 h and 72 h after treatment application. For the Solonetz, samples were collected 72 h after treatment application only. Both soils were collected from 0-10 cm depth, passed through a 2.4 mm sieve and are described in detail elsewhere (Liu et al., 2016a; Liu et al., 2016b) (Table S1). To determine whether SA led to any direct effects on soil microorganisms, two additional unplanted trays were included for each soil. All trays were transferred to a controlled environment chamber (Percival Scientific, Boone, IA, USA) maintained at 20°C with a 12 h photoperiod set to a light intensity of 150 mmol m^{-2} s^{-1}. Plants were watered once every two days with 15 mL water per well. The positions of the trays were changed daily.

2.2 SA treatment

A 5 mM SA solution was prepared by adding 13.8 mg SA (Sigma) in 1 mL of ethanol to 20 mL of ultrapure water. After 10 days growth (*i.e.* the two-leaf stage), 90 plants per soil were sprayed with 21 mL of 5 mM SA solution such that droplets visibly covered the shoot surfaces. The other 90 plants per soil were sprayed with 21 mL of a control solution, containing 1 mL of ethanol in 20 mL of ultrapure water. Similarly, half of the unplanted soils were sprayed with 21 mL of the 5 mM SA solution, while the other half was sprayed with the same volume of the control solution.

2.3 Sample collection

Each biological replicate comprised 30 plants by combining the three plants in each of 10 random wells per treatment. For the Calcisol, samples were collected from three regions (bulk soil, rhizosphere soil and root endophytic compartments) at two time points (48 h and 72 h post treatment application). For the Solonetz, samples were collected from two regions (bulk and rhizosphere soil) at one time point (72 h post treatment application). Bulk soil samples were collected in sterile plastic tubes and then transferred to -80ºC storage. Rhizosphere soil samples were collected by removing the roots of each plant from its pot, shaking manually to remove loosely adhered soil, and then shaking in sterile 50 mL tubes containing 25 mL of sterile 0.1 M phosphate

buffer (pH 7.0) at 250 rpm for 5 min. After shaking, roots were transferred to new tubes and the rhizosphere soil was pelleted by centrifugation at 12,000 g for 3 min and stored at -80°C after discarding the supernatant. For root endophytic samples, the roots were: 1) washed three times with distilled water and 0.1% Silwet L-77 in phosphate buffer (Horton et al., 2014), 2) sonicated at 20 kHz for 5 min to remove rhizoplane microorganisms (Bulgarelli et al., 2012), 3) washed in sterile phosphate buffer, 4) air dried, 5) ground in liquid nitrogen and then 6) stored at -80°C (Fig. S1). To check the efficiency of this process, a 200 µL aliquot of the last phosphate buffer wash was plated on nutrient agar, and did not lead to the growth of colony forming units within three days. To confirm that the SA treatment activated the SA pathway, shoot samples were collected, immediately snap frozen in liquid nitrogen and then stored at -80°C.

2.4 Quantification of SA pathway-associated gene transcripts

Based on an initial screen (Fig. S1), we selected *WCI2* and *WCI3* as marker genes to indicate whether SA treatment led to significant activation of the SA pathway. Real-time PCR was used to quantify the transcripts of these two genes relative to the 18S rRNA gene. To this end, frozen shoot samples were ground in liquid nitrogen with a mortar and pestle. RNA was then isolated using the SV Total RNA Isolation Kit (Promega) and cDNA was synthesized from 1.5 µg RNA in a 20 µL reaction using the Superscript III kit (Life Technologies), as instructed by the respective manufacturers. Relative quantification of mRNA transcripts was performed using a SYBR Green RT-PCR mixture on a ViiA™ 7 Real-Time PCR system (Applied Biosystems, USA). Primer sequences are shown in Table S2. Data processing was performed using the ViiA 7 RUO Software package (Applied Biosystems).

2.5 DNA extraction

DNA was extracted from 0.25-0.50 g soil using the Powersoil Kit (MOBIO Laboratories, CA) according to the manufacturer's instructions. For root samples, DNA was extracted from 0.2 g plant tissue using a CTAB method (Porebski et al., 1997). DNA concentration was determined using a Qubit™

fluorometer with Quant-iT dsDNA BR Assay Kits (Invitrogen) and then normalised to 2.5 ng μL^{-1} and 10 ng μL^{-1} for soil and plant samples, respectively.

2.6 Phylogenetic marker gene sequencing

Bacterial and archaeal 16S rRNA genes were amplified by PCR in 25 µL reactions containing 5.0 µL 5X Phire buffer (Thermo Fisher Scientific), 1.25 µL 10 µM dNTP (Invitrogen), 1.25 µL 10 µM reverse primer, 1.25 µL 10 µM forward primer, 0.5 µL Phire® Hot Start II and 15.75 µL molecular biology grade water. Bulk soil and rhizosphere samples were amplified using the primers 926F (5'-AAA CTY AAA KGA ATT GRC GG-3'; Engelbrektson *et al.* 2010) and 1392wR (3'-ACG GGC GGT GWG TRC-5'; Peiffer et al., 2013). Root endophytes were amplified using 799F (5'-AAC MGG ATT AGA TAC CCK G-3'; Horton et al., 2014) and 1193R (5'-ACG TCA TCC CCA CCT TCC-3'; Horton et al., 2014) as these primers reduce co-amplification of plant DNA (Horton et al., 2014). As all Solonetz samples were sequenced using the 454 GS FLX platform, the forward and reverse primers were modified on the 5' end to contain the 454 FLX Titanium Lib L adapters B and A, respectively. The reverse primers also contained a 5 to 6 base barcode sequence positioned between the primer sequence and the adapter. A unique barcode was used for each sample. All Calcisol samples were sequenced using the Illumina MiSeq platform. Consequently, the forward and reverse primers were modified on the 5' end to contain the Illumina overhang adapter for compatibility with the P5 and i7 Nextera XT indices, respectively. Thermocycling conditions were as follows: 98°C for 5 min; then 35 cycles of 98°C for 15 s, 55°C for 15 s and 72°C for 45 s; followed by 72°C for 7 min. Three amplifications were performed per sample and pooled to minimise PCR bias and no-template controls were included in all runs to check for contamination.

After size examination on 1.5% agarose gels: 1) Solonetz-associated amplicons were purified using Wizard® SV Gel and PCR Clean-Up kits (Promega), adjusted to 50 ng DNA μL^{-1}, pooled and then ran on a 454 GS FLX sequencer, and 2) Calcisol-associated amplicons were purified using Agencourt AMPure magnetic beads and subjected to dual indexing using the Nextera XT Index Kit (Illumina) as

per the manufacturer's instructions. Indexed Calcisol-associated amplicons were purified using Agencourt AMPure XP beads and then quantified using a PicoGreen dsDNA Quantification Kit (Invitrogen). Equal concentrations of each sample were pooled and sequenced on an Illumina MiSeq using 30% PhiX Control v3 (Illumina) and a MiSeq Reagent Kit v3 (600 cycle; Illumina) according the manufacturer's instructions.

2.7 Processing of sequence data

Solonetz 454 data were processed as previously described (Liu et al., 2017a), *viz*. sequences were quality filtered and dereplicated using the QIIME script split_libraries.py with the homopolymer filter deactivated (Caporaso et al., 2010), checked for chimeras against the GreenGenes database (10_2013 release) (DeSantis et al., 2006) using UCHIME ver. 3.0.617 (Edgar et al., 2011), homopolymer error corrected using Acacia (Bragg et al., 2012), clustered into operational taxonomic units at 97% using UCLUST v. 1.2.22 (Edgar, 2010) and then assigned GreenGenes taxonomy using BLAST+ v. 2.2.30. For the Calcisol MiSeq data, primers were removed using the QIIME v1.9.1 script multiple_extract_barcodes.py (Caporaso et al., 2010), sequence headers were modified to contain sample IDs using a custom bash script, then each file was quality filtered using the QIIME script multiple_split_libraries.py with the homopolymer filter deactivated. The forward reads from each sample were then concatenated into a single file and the checked for chimeras, clustered into OTUs and assigned taxonomy as described for the 454 data. The number of sequences per sample was rarefied to 1,900 for the Solonetz, 5,200 for the Calcisol bulk and rhizosphere soil and 8,450 for Calcisol wheat root endophytes. The mean number of observed (Sobs) Operational Taxonomic Units (OTUs) and Simpson's diversity index values were calculated using QIIME.

2.8 Statistical analyses

Differences in the observed numbers of taxa, Simpson's Diversity Index and $\log_{10}$ WCI2 and WCI3 transcript abundances were identified using analysis of variance (ANOVA) with Tukey's HSD tests for post-hoc comparisons of means. The effects of SA treatment on the composition of bacterial

communities were investigated using Permutational Multivariate Analysis of Variance (PERMANOVA) after Hellinger transformation of the OTU abundances (Legendre and Gallagher, 2001). All analyses were performed using R v.2.12.0.

3. Results and discussion

3.1 Activation of the wheat SA signalling pathway

The abundances of *WCI2* and *WCI3* transcripts were significantly and positively associated with SA treatment in both soil types (Fig. 1). In the Calcisol, 48 h after SA treatment, the abundance of *WCI2* and *WCI3* gene transcripts significantly increased by 8-fold and 165-fold relative to the control, respectively (Fig. 1). Similarly, in the Calcisol, 72 h after SA treatment, the abundance of *WCI2* and *WCI3* gene transcripts significantly increased by 8- and 56-fold, respectively (Fig. 1). In the Solonetz, 72 h after SA treatment, the abundance of *WCI2* and *WCI3* gene transcripts significantly increased by 47-fold and 95-fold relative to the control, respectively (Fig. 1). These results indicate that the exogenous SA treatment successfully activated the SA signalling pathway of wheat grown in both soil types (Gorlach et al., 1996; Sardesai et al., 2005).

3.2 Differences in the diversity of bulk soil and root-associated microbial communities

Calcisol and Solonetz bulk soil and rhizosphere microbial communities were dominated by members of the Chrenarchaeota, Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi and Proteobacteria (Figs. 2 and 3). In the Calcisol, the composition of microbial communities differed significantly between bulk and rhizosphere soil ($P < 0.001$; PERMANOVA) as did the richness (Sobs, $P < 0.001$) and evenness (Simpson, $P < 0.001$) (Fig. 2). Root endophytic communities were dominated by members of the Actinobacteria and Proteobacteria in the Calcisol (Fig. 2b) and were not characterised in the Solonetz. As different primers were used to target root endophytes, formal comparisons with bulk soil and rhizosphere were limited. Nonetheless, for the Calcisol, our data indicate that the composition of root endophytic

communities differed considerably from those associated with bulk soil, and more subtly when compared with rhizosphere soil (Fig. 2a, b). In addition, for the Calcisol, our data indicate that the richness of endophytic communities was less than that of those associated with rhizosphere soils (Fig. 2c). For the Solonetz, microbial communities associated with bulk and rhizosphere soil had similar richness and evenness (Fig. 3b) but differed significantly in composition (P = 0.003, PERMANOVA; Fig. 3). These findings are consistent with a wide-range of previous studies that report differences in the diversity of bulk soil and root-associated microbial communities (Berendsen et al., 2012), and highlight that our methodological approach was adequately sensitive to detect known differences in microbial diversity.

3.3 Microbial diversity was not affected by exogenous SA treatment of wheat shoots

Importantly, SA treatment of wheat shoots did not lead to significant changes in the composition, richness or evenness of bulk and rhizosphere soil microbial communities in either soil type. Likewise, the composition, richness and evenness of root endophytic microbial communities were not affected by SA treatment.

Doornbos *et al*. (2011) investigated the impacts of SA signalling on the rhizosphere microbiome of *A. thaliana* by: 1) exogenously applying SA and its functional analogue benzothiadiazole, and 2) using two SA signalling-deficient mutants as well as one that over-produced SA. Consistent with our study, they found no effects of SA signalling on the diversity of rhizosphere bacteria communities (using denaturing gradient gel electrophoresis, DGGE). Similarly, Sonnemann et al. (2002) reported that aerial application of BION® (a commercial plant activator for induced resistance) on barley did not cause measurable changes in the free-living soil biota within three months (Sonnemann et al., 2002). In contrast to these, Hein et al. (2008) found significant differences in terminal restriction fragment length polymorphism (T-RFLP) profiles of bacterial communities between *A. thaliana* mutants that were constitutive and non-inducible for systemic acquired resistance, which is triggered by an

accumulation of SA. Furthermore, Lebeis et al. (2015) found that the roots of *A. thaliana* mutants that either have enhanced SA levels, or were unable to synthesise or sense SA were associated with root microbiomes that differed in composition at the class level, suggesting a role of SA in structuring the plant root microbiome.

In summary, data indicating a role of plant SA signalling in structuring root microbiomes currently derive solely from studies focussing on *A. thaliana* mutants. In contrast, studies focussing on the impacts of exogenous applications of SA indicate that its effects on root microbiomes, if any, are negligible. *Arabidopsis* mutants that are either compromised at or overexpressing SA signalling may have distinct physiological profiles such as altered developmental senescence and primary and secondary metabolite production (Carvalhais et al., 2015; Morris et al., 2001). Thus, an explanation for this difference may be that microbial communities associated with mutant plants are influenced by the physiology of their hosts since germination. This is not a situation that would be encountered in natural environments. On the other hand, the microbiomes of exogenously treated plants are exposed to relatively acute physiological changes that may only allow time for negligible changes in microbial diversity. Another explanation is that the genetic differences between SA mutants and the wild type may be associated with unknown phenotypic changes (*i.e.* those not related to SA signalling) due to the complex interconnectedness of metabolic pathways (Lu et al., 2008). While exogenously applied chemicals may also trigger untargeted responses, the genetic background of the plant remains constant and such chemicals are generally short lived. Alternatively, the root microbiome may respond to acute changes in JA (Carvalhais et al., 2013; Liu et al., 2017a), but not SA signalling, as a consequence of differences in the coevolution of plants and microbes. For example, while priming by microbes is well documented for the JA pathway, the roles of rhizosphere microbes in the context of SA signalling remain to be elucidated. Further studies are required to determine the extent to which plant defence signalling influences the microbiomes of other plant species, at various developmental stages, in different soils and under a wide-range of environmental conditions. Lastly, further studies should be conducted to determine whether there will be changes

in the root microbiomes after longer period of time post treatments. These results would complement ours by unravelling the effects of secondary plant responses after SA signalling on root microbiome composition.

3.4 Conclusion

In this study, we observed no significant rapid changes in the diversity of wheat root microbiomes in response to activation of the SA signalling pathway via exogenous application of SA in two soil types. This is the first study to investigate the influence of SA signalling on the root microbiome of a commercially relevant crop and indicates that application of SA may not be a successful approach to influence the root microbiome of wheat within the timeframe tested.

Acknowledgements

We gratefully acknowledge Mark Dieters for providing wheat seeds; Christian Forstner for performing the Illumina sequencing; Anna Balzer, Yash Dang and Mark Crawford for help with the soil collection; and the Australian Research Council for financial support (DP1094749).

Author contributions

HL, PMS, LCC and PGD conceived and designed the study. HL and LCC performed the experiments. PGD and HL analysed the data and wrote the manuscript with input from all authors.

Competing financial interests

The authors declare no conflict of interest.

Data accessibility

The 16S rRNA amplicon sequences associated with this study have been deposited in the NCBI SRA under accession: PRJNA356991.

References

Badri, D.V., Loyola-Vargas, V.M., Du, J., Stermitz, F.R., Broeckling, C.D., Iglesias-Andreu, L., Vivanco, J.M., 2008. Transcriptome analysis of *Arabidopsis* roots treated with signaling compounds: a focus on signal transduction, metabolic regulation and secretion. New Phytol. 179, 209-223.

Badri, D.V., and Vivanco, J.M., 2009. Regulation and function of root exudates. Plant Cell Environ. 32: 666-681.

Berendsen, R.L., Pieterse, C.M.J., Bakker, P.A.H.M., 2012. The rhizosphere microbiome and plant health. Trends Plant Sci. 17, 478–486.

Bragg, L., Stone, G., Imelfort, M., Hugenholtz, P., Tyson, G.W., 2012. Fast, accurate error-correction of amplicon pyrosequences using Acacia. Nat. Methods 9, 425–426.

Bulgarelli, D., Rott, M., Schlaeppi, K., Ver, E., Van Themaat, L., Ahmadinejad, N., Assenza, F., Rauf, P., Huettel, B., Reinhardt, R., Schmelzer, E., Peplies, J., Gloeckner, F.O., Amann, R., Eickhorst, T., Schulze-Lefert, P., 2012. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. Nature 488.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.

Carvalhais, L.C., Dennis, P.G., Badri, D. V., Tyson, G.W., Vivanco, J.M., Schenk, P.M., 2013. Activation of the jasmonic acid plant defence pathway alters the composition of rhizosphere bacterial communities. PLoS One 8, 1–5.

Carvalhais, L.C., Dennis, P.G., Badri, D. V, Kidd, B.N., Vivanco, J.M., Schenk, P.M., 2015. Linking

jasmonic acid signaling, root exudates, and rhizosphere microbiomes. Mol. Plant. Microbe. Interact. 28, 1049–58.

Carvalhais, L.C., Schenk, P.M., Dennis, P.G., 2017. Jasmonic acid signalling and the plant holobiont. Curr. Opin. Microbiol. 37, 42–47.

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microbiol. 72, 5069–72.

Doornbos, R.F., Geraats, B.P.J., Kuramae, E.E., Van Loon, L.C., Bakker, P.A.H.M., 2011. Effects of jasmonic acid, ethylene, and salicylic acid signaling on the rhizosphere bacterial community of *Arabidopsis thaliana*. Mol. Plant-Microbe Interact. 24, 395–407.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–1.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–200.

Engelbrektson, A., Kunin, V., Wrighton, K.C., Zvenigorodsky, N., Chen, F., Ochman, H., Hugenholtz, P., 2010. Experimental factors affecting PCR-based estimates of microbial species richness and evenness. ISME J. 4, 642–647.

Glazebrook, J., 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu. Rev. Phytopathol. 43, 205–227.

Gorlach, J., Volrath, S., Knauf-Beiter, G., Hengy, G., Beckhove, U., Kogel, K.H., Oostendorp, M., Staub, T., Ward, E., Kessmann, H., Ryals, J., 1996. Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. Plant Cell Online 8, 629–643.

Hein, J.W., Wolfe, G. V., Blee, K.A., 2008. Comparison of rhizosphere bacterial communities in *Arabidopsis thaliana* mutants for systemic acquired resistance. Microb. Ecol. 55, 333–343.

Horton, M.W., Bodenhausen, N., Beilsmith, K., Meng, D., Muegge, B.D., Subramanian, S., Vetter, M.M., Vilhjálmsson, B.J., Nordborg, M., Gordon, J.I., Bergelson, J., 2014. Genome-wide association study of *Arabidopsis thaliana* leaf microbial community. Nat. Commun. 5, 5320.

Lebeis, S.L., Paredes, S.H., Lundberg, D.S., Breakfield, N., Gehring, J., McDonald, M., Malfatti, S., del Rio, T.G., Jones, C.D., Tringe, S.G., Dangl, J.L., 2015. Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. Science 349, 860–864.

Legendre, P., Gallagher, E.D., 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129, 271–280.

Liu, H., Carvalhais, L.C., Crawford, M., Dang, Y.P., Dennis, P.G., Schenk, P.M., 2016a. Strategic tillage increased the relative abundance of Acidobacteria but did not impact on overall soil microbial properties of a 19-year no-till Solonetz. Biol. Fertil. Soils 52, 1021–1035.

Liu, H., Carvalhais, L.C., Rincon-Florez, V., Crawford, M., Dang, Y.P., Dennis, P.G., Schenk, P.M., 2016b. One-time strategic tillage does not cause major impacts on soil microbial properties in a no-till Calcisol. Soil Tillage Res. 158, 91–99.

Liu, H., Carvalhais, L.C., Schenk, P.M., Dennis, P.G., 2017a. Effects of jasmonic acid signalling on the wheat microbiome differ between body sites. Sci. Rep. 7, 41766.

Liu, H., Carvalhais, L.C., Crawford, M., Singh, E., Dennis, P.G., Pieterse, C.M., Schenk, P.M., 2017b. Inner plant values: diversity, colonization and benefits from endophytic bacteria. Front. Microbiol. 8.

Lu, Y., Savage, L.J., Ajjawi, I., Imre, K.M., Yoder, D.W., Benning, C., DellaPenna, D., Ohlrogge, J.B., Osteryoung, K.W., Weber, A.P., Wilkerson, C.G., Last, R.L., 2008. New connections across pathways and cellular processes: industrialized mutant screening reveals novel associations between diverse phenotypes in Arabidopsis. Plant Physiol. 146, 1482–1500.

Morris, K., -Mackerness, S.A.H., Page, T., John, C.F., Murphy, A.M., Carr, J.P., and Buchanan-Wollaston, V. (2000) Salicylic acid has a role in regulating gene expression during leaf senescence. Plant J. 23: 677-685.

Peiffer, J.A., Spor, A., Koren, O., Jin, Z., Tringe, S.G., Dangl, J.L., Buckler, E.S., Ley, R.E., 2013. Diversity and heritability of the maize rhizosphere microbiome under field conditions. Proc. Natl. Acad. Sci. U. S. A. 110, 6548–53.

Pieterse, C.M.J., Leon-Reyes, A., Van der Ent, S., Van Wees, S.C.M., 2009. Networking by small-molecule hormones in plant immunity. Nat. Chem. Biol. 5, 308–316.

Porebski, S., Bailey, L.G., Baum, B.R., 1997. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Mol. Biol. Report. 15, 8–15.

Sardesai, N., Subramanyam, S., Nemacheck, J., Williams, C.E., 2005. Modulation of defense-response gene expression in wheat during Hessian fly larval feeding. J. Plant Interact. 1, 39–50.

Schenk, P.M., Kazan, K., Wilson, I., Anderson, J.P., Richmond, T., Somerville, S.C., and Manners, J.M. (2000) Coordinated plant defense responses in Arabidopsis revealed by microarray analysis. Proc. Natl. Acad. Sci. U. S. A. 97: 11655-11660.

Sonnemann, I., Finkhaeuser, K., Wolters, V., 2002. Does induced resistance in plants affect the belowground community? Appl. Soil Ecol. 21, 179-185.

Figure captions

Fig. 1 The effect of salicylic acid (SA) application on the transcription of genes associated with the SA signalling pathway in the shoots of 10-day-old wheat seedlings grown in the (a) Calcisol, and (b) Solonetz soil. Asterisks indicate significant differences between control and SA treated plants ($P < 0.05^{*}$, $P < 0.01^{**}$, $P < 0.001^{***}$, ANOVA). Error bars represent standard deviations (n=3). To obtain raw values raise 10 to the power of the log values shown.

Fig. 2 Heatmap summarising the frequencies of dominant bacterial and archaeal populations (i.e. operational taxonomic units (OTU) that were present at ≥1% relative abundance in any sample) in response to SA treatment in Calcisol (a) bulk soil and rhizosphere soil communities, (b) root endophytic communities, and (c) the richness of bulk soil and root-associated microbial communities, over time (48 h and 72 h post-SA treatments).

Fig. 3 Heatmap summarising the frequencies of dominant bacterial and archaeal populations (i.e. operational taxonomic units (OTU) that were present at ≥1% relative abundance in any sample) in response to SA treatment in Solonetz bulk soil and rhizosphere soil communities, 72 h after SA treatment, and (b) the richness of bulk soil and root-associated microbial communities in the Solonetz.

Figr-1Figure 1

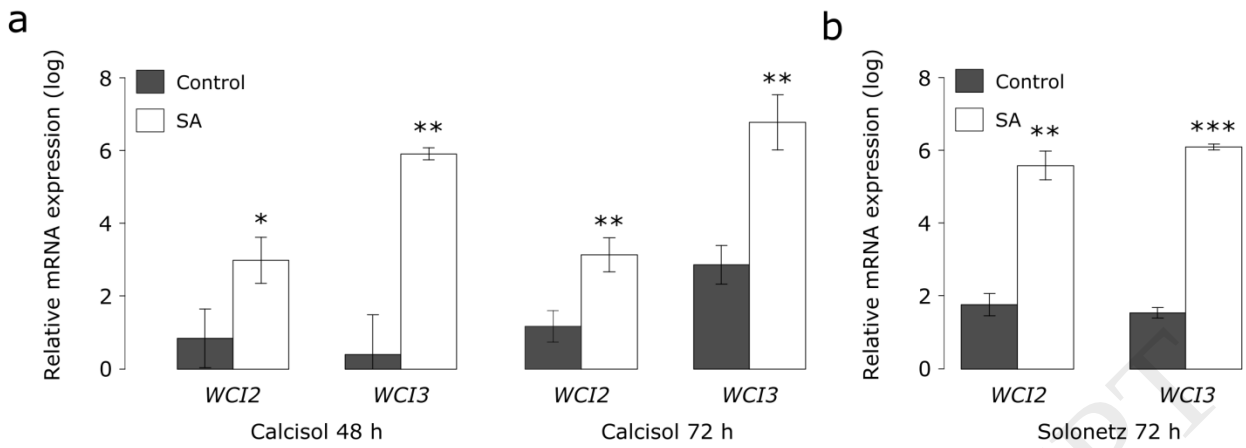

Figure 2

Calcisol

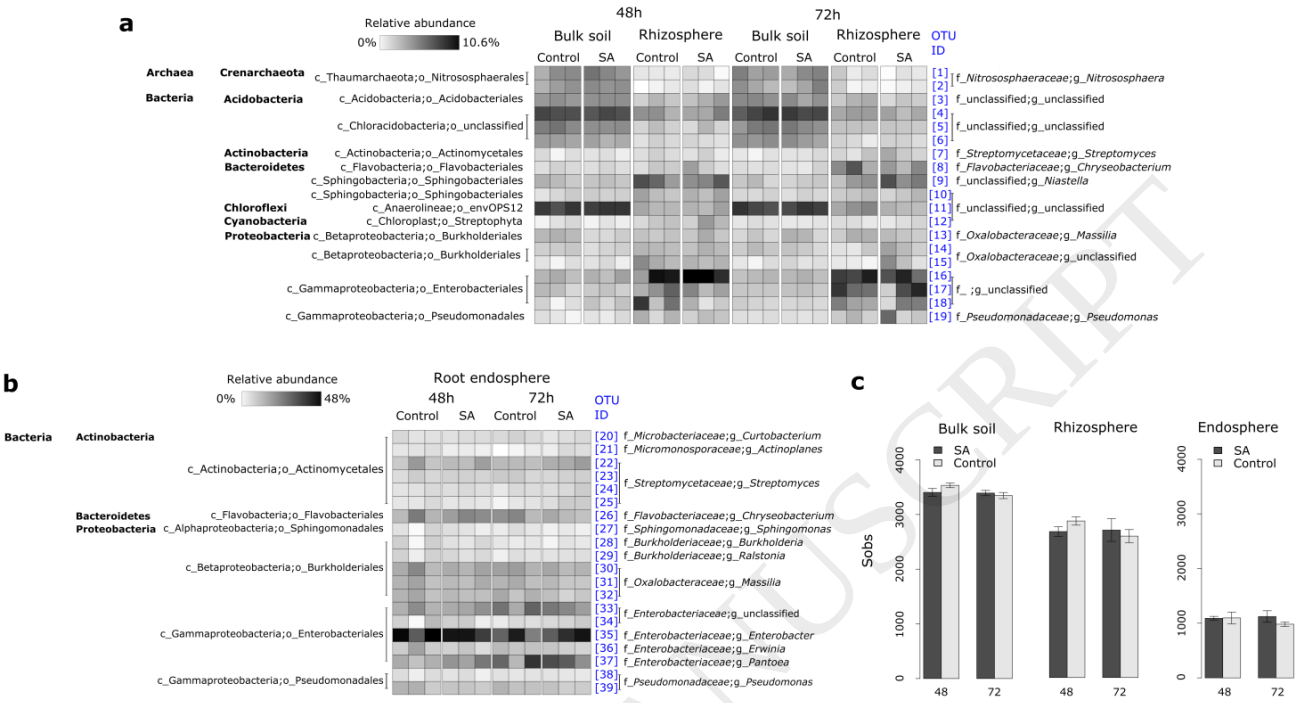

Figure 3

Solonetz