CrossMark

Plant defence inducers rapidly influence the diversity of bacterial communities in a potting mix

Lilia C. Carvalhais *, Paul G. Dennis, Peer M. Schenk

School of Agriculture and Food Sciences, The University of Queensland, Brisbane, 4072 QLD, Australia

ABSTRACT

Plant hormones and their functional analogues are used in agriculture to combat plant diseases and pests. These chemicals are generally targeted at shoots, but soils inevitably receive a dose during application. In this study, we used 16S rRNA gene amplicon pyrosequencing to determine whether the diversity of bacterial communities in a potting mix (referred here as 'model soil') is influenced by exposure to salicylic acid (SA), methyl jasmonate (MeJA), ethylene (ET) and abscisic acid (ABA). The number of operational taxonomic units (OTUs; richness) and Simpson's Diversity Index values (evenness) associated with each sample was not influenced by the hormone treatments, but changes in community composition were observed. Relative to the control, all hormones more than doubled the abundance of a *Limnobacter* population, ABA led to a four-fold increase in a *Cellvibrio* population, ET led to a c. 70% decrease in an *Algoriphagus* population, and MeJA and ABA applications halved the abundance of a *Massilia* population. These changes may influence plant productivity.

© 2014 Published by Elsevier B.V.

1. Introduction

Plant diseases are estimated to cause up to 42% yield losses for the eight most commonly grown crops worldwide (Oerke et al., 1994; Fletcher et al., 2006; Fisher et al., 2012). The causal agents of these diseases are typically combatted with pesticides, breeding techniques for the development of resistant crops, and chemicals that induce systemic plant resistance (Tamm et al., 2011; Ghimire and Woodward, 2013). The latter approach enhances the plant's innate defence mechanisms (Tamm et al., 2011), which can be induced by chemicals such as salicylic acid (SA; Lee et al., 2009; Vlot et al., 2009), functional analogues of SA (e.g Bion®; Friedrich et al., 1996; Gorlach et al., 1996; Sparla et al., 2004; Bokshi et al., 2008), ethylene (ET; van Loon et al., 2006), abscisic acid (ABA; Mauch-Mani and Mauch, 2005) and jasmonic acid (JA; Cohen et al., 1993). SA and ABA can be applied in solution by spraying; however, ET and JA (in the form of methyl-JA, MeJA) are mostly applied as gases or volatiles (Jin et al., 2009; Nahar et al., 2011). These chemicals are generally targeted at shoots, but, during application, the soil typically receives a dose.

Previously we demonstrated that treatment of *Arabidopsis* plants with MeJA alters the composition of rhizosphere bacterial communities via activation of the jasmonic acid signalling pathway, but direct effects of MeJA on the diversity of bacteria associated with bulk soil were not detected (Carvalhais et al., 2013a). Effects of SA, ABA, and ET on soil microbial communities have not been investigated; however, ethylene has been shown to strongly influence the composition of bacterial communities associated with zeolite biofilters in air treatment systems (Fu et al., 2011). Given the important roles performed by soil bacteria in plant nutrition, disease suppression and other soil ecosystem functions (Carvalhais et al., 2013b,c; Nannipieri et al., 2003), it is important to improve understanding of the effects of plant hormones on soil bacterial diversity.

In this study, we investigated changes in bacterial diversity of a model soil (potting mix) in response to the plant hormones SA, MeJA, ABA, and ET using 16S rRNA gene amplicon pyrosequencing. Although bacterial richness and evenness were unaffected, distinct changes in bacterial community composition were revealed.

2. Materials and methods

2.1. Plant hormone amendment of a model soil

After three weeks incubation at 24 °C with a light intensity of 150 μmol m^{-2} s^{-1} in a controlled environment chamber (Percival Scientific, Boone, IA, USA), a potting mix (Greenfingers B2, Nerang,

* Corresponding author at: The University of Queensland, Mansfield Place, John Hines Building, 5th floor, Saint Lucia 4072, Australia. Tel.: +61 7 334 69948; fax: +61 7 3365 1699.
E-mail address: l.carvalhais@uq.edu.au (L.C. Carvalhais).

Australia) was amended with salicylic acid (SA), abscisic acid (ABA), methyl jasmonate (MeJA), and ethylene (ET). We opted to use a potting mix as a 'model soil' as it is routinely used in horticulture as a plant-supporting substrate. SA and ABA were applied as liquid sprays, while MeJA and ET were delivered in the volatile/gaseous form. Briefly, SA and ABA treatments were applied by spraying a 5 mM solution of SA and a 100 μM solution of ABA dissolved in 5% and 0.1% ethanol, respectively. For the MeJA treatment application, a cottonwool ball containing 300 μL of a 0.5% MeJA solution in ethanol was taped to the inner wall of the plastic lid of a 20 L container and then the tray was wrapped with two transparent plastic bags. The ET treatment was applied by injecting a 4 mL volume of ET gas into a 20 L container where the model soil was kept in an air-sealed system. The control model soil as well as each treatment were treated in exactly the same way as the other hormone treatments, but MeJA, SA, ABA, or ET was not added. This included a cotton ball with ethanol taped onto the wall of the chamber as well as spraying with 5% ethanol. These mock treatments were applied to all experiments to allow direct comparisons. A total of three biological replicates were analysed. Each biological replicate comprised a pool of two model soil samples collected from different locations within the tray where the treatments were applied. During liquid spray applications, eight–twelve leaf shoots of *Arabidopsis thaliana* ecotype Col-0 were placed on the model soil surface to ensure that the exposed area would mimic incidental spraying. Shoots were then removed immediately after the application of treatments.

2.2. Sample collection, DNA extraction and amplification of 16S rRNA genes

Samples of the model soil were collected with a 12 mm borer 72 h after hormonal treatments and stored in Lifeguard™ Soil Preservation Solution (MO BIO Laboratories, Carlsbad, CA) at $-20\,^{\circ}$C. Total DNA was extracted from these samples using the PowerSoil® DNA Isolation kit according to the manufacturer's instructions (MO BIO Laboratories, Carlsbad, CA). The quality of the extracted DNA was assessed on a 1% agarose gel. Extracted DNA concentrations were determined using a Qubit™ fluorometer with Quant-iT dsDNA BR Assay Kits (Invitrogen, Carlsbad, CA) and then normalised to 10 ng μl^{-1}. Universal 16S rRNA genes were amplified by PCR in 50 μl volumes containing 20 ng DNA, molecular biology grade water, 1X PCR buffer minus Mg, 50 nM of each of the dNTPs, 1.5 mM MgCl$_2$, 0.3 mg BSA, 0.02 U *Taq* DNA Polymerase, 8 μM each of the primers 803F and 1392R modified on the 5′ end to contain the 454 FLX Titanium Lib L adapters B and A, respectively (Engelbrektson et al., 2010). These primers amplify both bacteria and archaea. The reverse primers also contained a 5–6 base barcode sequence positioned between the primer sequence and the adapter. A unique bar-code was used for each sample. Thermocycling conditions were as follows: 95 °C for 3 min; then 30 cycles of 95 °C for 30 s, 55 °C for 45 s, 72 °C for 90 s; and then 72 °C for 10 min. Amplicons were purified using a QIAquick PCR purification kit (Qiagen, Hilden, Germany), quantified using a Qubit™ fluorometer with a Quant-iT dsDNA BR Assay Kit and then normalised to 25 ng μl^{-1} and pooled for 454 pyrosequencing. Sequencing was performed by the Australian Centre for Ecogenomics at the University of Queensland (Brisbane, Australia).

2.3. Data analyses

Sequence data were processed as described in (Dennis et al., 2013). Briefly, sequences were quality filtered and dereplicated using the QIIME script split_libraries.py with the homopolymer filter deactivated (Caporaso et al., 2010) and then checked for chimeras against the GreenGenes database (De Santis et al., 2006)

using UCHIME ver. 3.0.617 (Edgar et al., 2011). Homopolymer errors were corrected using Acacia (Bragg et al., 2012). Sequences were then subjected to the following procedures using QIIME scripts with the default settings: (1) sequences were clustered at 97% similarity, (2) cluster representatives were selected, (3) Greengenes taxonomy was assigned to the cluster representatives using BLAST, (4) tables with the abundance of different operational taxonomic units (OTUs) and their taxonomic assignments in each sample were generated. The number of reads was then normalised to 1950 per sample by re-sampling the OTU table. The mean number of OTUs (observed richness) and Simpson's Diversity Index values (Simpson, 1949) corresponding to 1950 sequences per sample were calculated using QIIME. Differences in these diversity metrics were investigated using one-way ANOVA ($P < 0.05$) followed by Tukey's post-hoc tests. Differences in the composition of microbial communities were investigated using principal component analysis. The most discriminating OTUs between treatments were subjected to separate one-ways ANOVAs with post-hoc Tukey tests to determine whether they differed between treatments. All OTU abundances were Hellinger transformed prior to analysis and all analyses were implemented using R (Legendre and Gallagher, 2001).

2.4. Confirmation that the hormone concentrations were adequate to induce plant defence signalling pathways

Chemical concentrations were chosen based on previous studies (Schenk et al., 2000; Campbell et al., 2003). To confirm that these concentrations were sufficient to induce plant MeJA, SA, ET, and ABA signalling, we conducted an independent experiment in which the expression levels of marker genes characteristic of each pathway were quantified using real-time PCR. Plant growth conditions as well as MeJA, SA, ABA, and ET treatments were carried out as described previously. A total of 160 wild-type *Arabidopsis thaliana* (Col-0) plants were cultivated in potting mix within a controlled environment chamber (Percival Scientific, Boone, IA, USA) at 24 °C with a light intensity of 150 μmol m^{-2} s^{-1}. Treatments were applied when plants developed 8–12 leaves, which were then harvested 72 h later, except for the ET treatment, in which plants were harvested 24 h later. Two trays containing 60 plants each were used per treatment. As for the plantless experiment, the tray positions within the growth chamber were also changed daily throughout the experiment. Three biological replicates containing a pool of approximately 40 plant shoots each were used. Expression of *VSP2* and *PDF1.2* was measured for JA; *PR1* and *PR5* for SA; *ERF1*, *PR4*, *CHI-B*, and *ORA59* for ET; as well as *RD22* and *RD29B* for ABA. Transcript levels were normalised to the expression of a mixture of three genes encoding β-*ACTIN2*, *ACTIN7*, and *ACTIN8* (Schenk et al., 2005). A list of primer sequences and target genes can be found in the Supplementary Table S1. Results for the gene expression analysis were included as Supplementary Fig. S1.

3. Results and discussion

At the phylum level, all samples were dominated by *Proteobacteria*, *Bacteroidetes* and *Firmicutes*, which represented $65 \pm 5.8\%$, $22.2 \pm 5.3\%$ and $8.4 \pm 2.1\%$ of the community (mean ± standard deviation), respectively (Fig. 1). No archaeal sequences were detected. At the operational taxonomic unit (OTU) level, the proteobacterial populations included representatives of the alpha-, beta-, delta- and gammaproteobacterial classes, while the *Bacteroidetes* and *Firmicutes* were dominated by members of the *Sphingobacteria* and *Bacilli*, respectively (Fig. 1). The most dominant OTUs in all samples were a *Pseudomonas*, a *Limnobacter*, a *Comamonadaceae* and an *Algoriphagus* population (Fig. 1). These
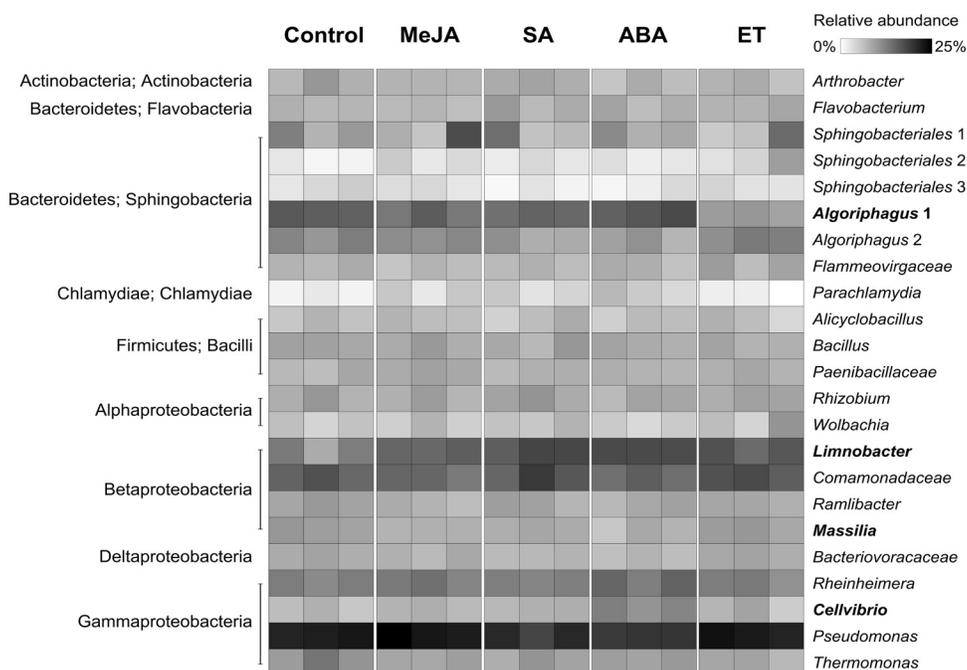
Fig. 1. Heatmap summarising the abundance of operational taxonomic units (OTU) that were present at more than 1% relative abundance in at least one sample. OTUs that were significantly different relative to the control are highlighted in bold font. All data were Hellinger transformed prior to analysis. MeJA = methyl jasmonate, SA = salicylic acid, ABA = abscisic acid, ET = ethylene.
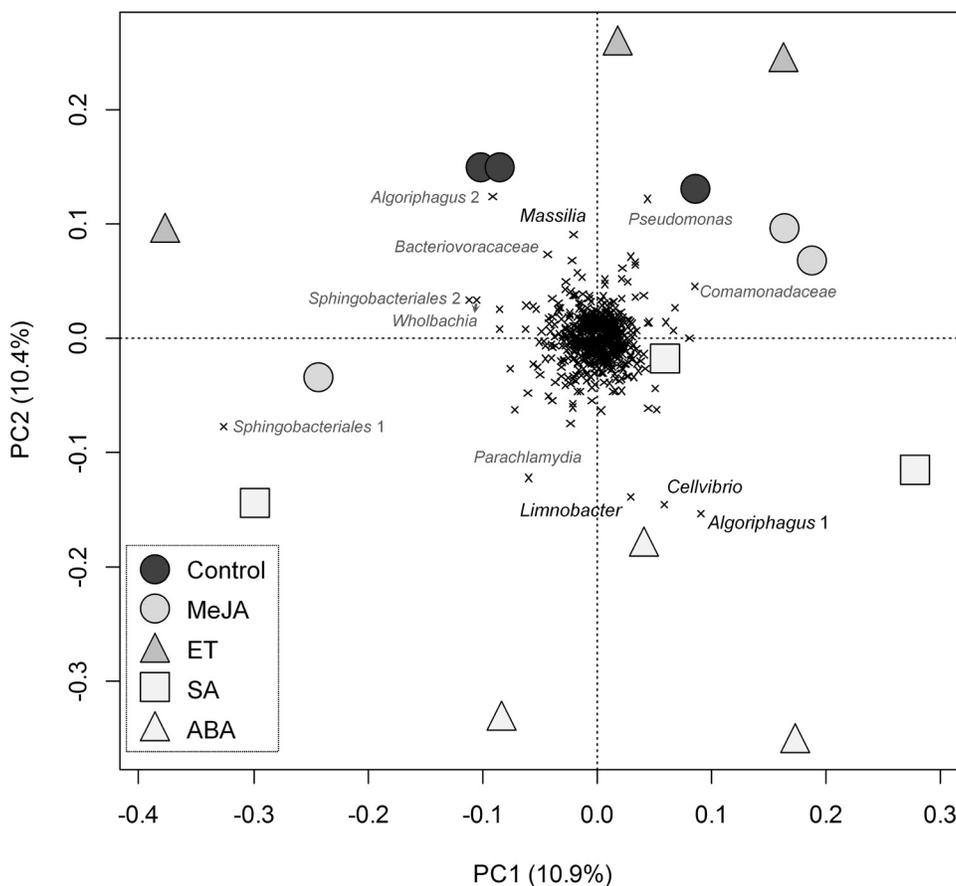
Fig. 2. Principal component analysis summarising differences in the composition of bacterial communities associated with control and hormone treatments. The OTUs highlighted in bold were significantly influenced by at least one hormone treatment. MeJA = methyl jasmonate, SA = salicylic acid, ABA = abscisic acid, ET = ethylene.
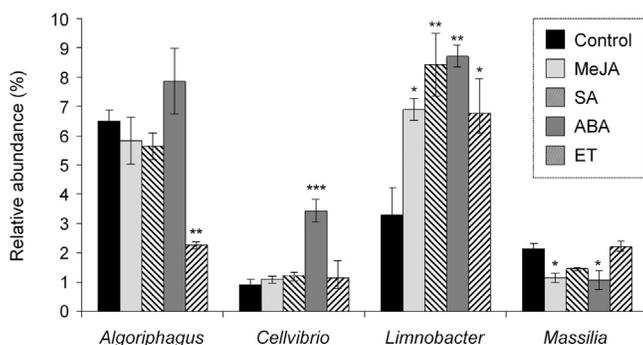
bacteria have been observed in association with plant and soil environments and, with the exception of the *Algoriphagus* population, have been implicated in plant growth promotion (Vyas and Gulati, 2009; Stout et al., 2010; Li et al., 2012; Ubalde et al., 2012; Liu et al., 2013; Shen et al., 2013). The number of OTUs and Simpson's Diversity Index values associated with each sample was not influenced by the hormone treatments (Supplementary Table S2); however, principal component analysis (PCA) revealed a number of OTUs that differed in abundance between samples (Fig. 2). An *Algoriphagus*, a *Limnobacter*, a *Cellvibrio* and a *Massilia* population were significantly influenced by the hormone applications (*P* < 0.05, ANOVA). Other populations, however, were associated with individual samples only (Figs. 1 and 2).

Relative to the control, all hormones treatments more than doubled the abundance of a *Limnobacter* population (Fig. 3). Within this genus, *Limnobacter thiooxidans* has been shown to inhibit the growth of *A. thaliana* via an unidentified mechanism (Blom et al., 2011). An enrichment of *Limnobacter* populations in soil resulting from inadvertent plant hormone treatment could, therefore, negatively influence plant growth.

In contrast, *Cellvibrio* populations have been reported to be positively associated with wheat shoot biomass (Anderson and Habiger, 2012). ABA application led to a four-fold increase in a *Cellvibrio* population (Fig. 3). This suggests that hormone treatment of soil can induce changes in microbial community composition that may influence plant productivity in both positive and negative ways.

We observed a c. 70% decrease in an *Algoriphagus* population in response to ET treatment of a model soil. Previous studies have shown that ET influences soil microbial processes such as methanotrophy and nitrification (Porter, 1992; Jackel et al., 2004); however, our results are the first to demonstrate effects on specific bacterial populations in a potting mix. In air biofiltration systems, ethylene appears to enrich specific bacterial populations that may degrade ethylene (Fu et al., 2011). Our results indicate that ET does not enrich specific bacterial populations in the model soil used in our experiments.

MeJA and ABA application halved the abundance of a *Massilia* population (Fig. 3). Members of this genus have been shown to colonise plant seeds, leaves and roots (Ofek et al., 2012; Bodenhausen et al., 2013). Their influence on plant productivity, however, remains unclear. A lower abundance of *Massilia* populations has been observed in disease-suppressive relative to non-suppressive soils (Klein et al., 2013). Nonetheless, an increase in soil suppressiveness has been achieved upon addition of chitin

to soil despite a concomitant increase in the abundance of *Massilia* species (Cretoiu et al., 2013). There is mixed evidence, therefore, concerning the relationship between plant productivity and the prevalence of *Massilia* populations.

In conclusion, our study demonstrates in principle that plant hormones can influence the composition of bacterial communities in a model soil. These changes may influence plant productivity and other ecosystem services and as such require further investigation.

Acknowledgement

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2014.06.011.

References

Anderson, M., Habiger, J., 2012. Characterization and identification of productivity-associated rhizobacteria in wheat. Appl. Environ. Microb. 78, 4434–4446.

Blom, D., Fabbri, C., Connor, E.C., Schiestl, F.P., Klauser, D.R., Boller, T., Eberl, L., Weisskopf, L., 2011. Production of plant growth modulating volatiles is widespread among rhizosphere bacteria and strongly depends on culture conditions. Environ. Microbiol. 13, 3047–3058.

Bodenhausen, N., Horton, M.W., Bergelson, J., 2013. Bacterial communities associated with the leaves and the roots of *Arabidopsis thaliana*. PLoS One 8, e56329.

Bokshi, A.I., Jobling, J., McConchie, R., 2008. A single application of Milsana (R) followed by Bion (R) assists in the control of powdery mildew in cucumber and helps overcome yield losses. J. Hortic. Sci. Biotech. 83, 701–706.

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.

Campbell, E.J., Schenk, P.M., Kazan, K., Penninckx, I.A.M.A., Anderson, J.P., Maclean, D.J., Cammue, B.P.A., Ebert, P.R., Manners, J.M., 2003. Pathogen-responsive expression of a putative ATP-binding cassette transporter gene conferring resistance to the diterpenoid sclareol is regulated by multiple defense signaling pathways in *Arabidopsis*. Plant Physiol. 133, 1272–1284.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, 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., Tumbaugh, 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., 2013a. Activation of the jasmonic acid plant defence pathway alters the composition of rhizosphere bacterial communities. PLoS One 8, e56457.

Carvalhais, L.C., Dennis, P.G., Fan, B., Fedoseyenko, D., Kierul, K., Becker, A., von Wiren, N., Borriss, R., 2013b. Linking plant nutritional status to plant–microbe interactions. PLoS One 8, e68555.

Carvalhais, L.C., Muzzi, F., Tan, C.H., Hsien-Choo, J., Schenk, P.M., 2013c. Plant growth in *Arabidopsis* is assisted by compost soil-derived microbial communities. Front Plant Sci. 4, 235.

Cohen, Y., Gisi, U., Niderman, T., 1993. Local and systemic protection against *Phytophthora infestans* induced in potato and tomato plants by jasmonic acid and jasmonic methyl ester. Phytopathology 83, 1054–1062.

Cretoiu, M.S., Korthals, G.W., Visser, J.H., van Elsas, J.D., 2013. Chitin amendment increases soil suppressiveness toward plant pathogens and modulates the actinobacterial and oxalobacteraceal communities in an experimental agricultural field. Appl. Environ. Microbiol. 79, 5291–5301.

De Santis, 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, 5069–5072.

Dennis, P.G., Guo, K., Imelfort, M., Jensen, P., Tyson, G.W., Rabaey, K., 2013. Spatial uniformity of microbial diversity in a continuous bioelectrochemical system. Bioresource Technol. 129, 599–605.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200.

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.

Fisher, M.C., Henk, D.A., Briggs, C.J., Brownstein, J.S., Madoff, L.C., McCraw, S.L., Gurr, S.J., 2012. Emerging fungal threats to animal, plant and ecosystem health. Nature 484, 186–194.

Fig. 3. Relative abundances of bacterial OTUs that were significantly influenced by plant hormones. The error bars represent standard deviations and the asterisks indicate that a particular treatment was significantly different to the control according to Tukey's Honest Significant Differences tests, where *P* < 0.05 (*), *P* < 0.01 (**) and *P* < 0.001 (***). MeJA = methyl jasmonate, SA = salicylic acid, ABA = abscisic acid, ET = ethylene.

Fletcher, J., Bender, C., Budowle, B., Cobb, W.T., Gold, S.E., Ishimaru, C.A., Luster, D., Melcher, U., Murch, R., Scherm, H., Seem, R.C., Sherwood, J.L., Sobral, B.W., Tolin, S.A., 2006. Plant pathogen forensics: capabilities, needs, and recommendations. Microbiol. Mol. Biol. 70, 450–471 R.

Friedrich, L., Lawton, K., Ruess, W., Masner, P., Specker, N., Rella, M.G., Meier, B., Dincher, S., Staub, T., Uknes, S., Metraux, J.P., Kessmann, H., Ryals, J., 1996. A benzothiadiazole derivative induces systemic acquired resistance in tobacco. Plant J. 10, 61–70.

Fu, Y.M., Shao, L.Z., Tong, L., Liu, H., 2011. Ethylene removal efficiency and bacterial community diversity of a natural zeolite biofilter. Bioresource Technol. 102, 576–584.

Ghimire, N., Woodward, R.T., 2013. Under- and over-use of pesticides: an international analysis. Ecol. Econ. 89, 73–81.

Gorlach, J., Volrath, S., KnaufBeiter, 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 8, 629–643.

Jackel, U., Schnell, S., Conrad, R., 2004. Microbial ethylene production and inhibition of methanotrophic activity in a deciduous forest soil. Soil. Biol. Biochem. 36, 835–840.

Jin, P., Zheng, Y.H., Tang, S.S., Rui, H.J., Wang, C.Y., 2009. Enhancing disease resistance in peach fruit with methyl jasmonate. J. Sci. Food Agr. 89, 802–808.

Klein, E., Ofek, M., Katan, J., Minz, D., Gamliel, A., 2013. Soil suppressiveness to *Fusarium* disease: Shifts in root microbiome associated with reduction of pathogen root colonization. Phytopathology 103, 23–33.

Lee, S., Hong, J.C., Jeon, W.B., Chung, Y.S., Sung, S., Choi, D., Joung, Y.H., Oh, B.J., 2009. The salicylic acid-induced protection of non-climacteric unripe pepper fruit against *Colletotrichum* gloeosporioides is similar to the resistance of ripe fruit. Plant Cell Rep. 28, 1573–1580.

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

Li, D., Rothballer, M., Engel, M., Hoser, J., Schmidt, T., Kuttler, C., Schmid, M., Schloter, M., Hartmann, A., 2012. Phenotypic variation in *Acidovorax radicis* N35 influences plant growth promotion. FEMS Microbiol. Ecol. 79, 751–762.

Liu, Y., Zuo, S., Zou, Y.Y., Wang, J.H., Song, W., 2013. Investigation on diversity and population succession dynamics of endophytic bacteria from seeds of maize (*Zea mays* L., Nongda108) at different growth stages. Ann. Microbiol. 63, 71–79.

Mauch-Mani, B., Mauch, F., 2005. The role of abscisic acid in plant–pathogen interactions. Curr. Opin. Plant Biol. 8, 409–414.

Nahar, K., Kyndt, T., De Vleesschauwer, D., Hofte, M., Gheysen, G., 2011. The jasmonate pathway is a key player in systemically induced defense against root knot nematodes in rice. Plant Physiol. 157, 305–316.

Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. Eur. J. Soil Sci. 54, 655–670.

Oerke, E.C., Dehne, D.W., Schonbeck, F., Weber, A., 1994. Crop Production and Crop Protection: Estimated Losses in Major Food and Cash Crops. Elsevier, Amsterdam.

Ofek, M., Hadar, Y., Minz, D., 2012. Ecology of root colonizing *Massilia* (Oxalobacteraceae). PLoS. One 7, e40117.

Porter, L.K., 1992. Ethylene inhibition of ammonium oxidation in soil. Soil Sci. Soc. Am. J. 56, 102–105.

Schenk, P.M., Kazan, K., Rusu, A.G., Manners, J.M., Maclean, D.J., 2005. The SEN1 gene of Arabidopsis is regulated by signals that link plant defense responses and senescence. Plant Physiol. Biochem. 43, 997–1005.

Shen, X.M., Hu, H.B., Peng, H.S., Wang, W., Zhang, X.H., 2013. Comparative genomic analysis of four representative plant growth-promoting rhizobacteria in *Pseudomonas*. BMC Genomics 14, 271.

Simpson, E.H., 1949. Measurement of species diversity. Nature 163, 688.

Sparla, F., Rotino, L., Valgimigli, M.C., Pupillo, P., Trost, P., 2004. Systemic resistance induced by benzothiadiazole in pear inoculated with the agent of fire blight (*Erwinia amylovora*). Sci. Hortic-Amsterdam 101, 269–279.

Stout, L.M., Dodova, E.N., Tyson, J.F., Nusslein, K., 2010. Phytoprotective influence of bacteria on growth and cadmium accumulation in the aquatic plant *Lemna minor*. Water Res. 44, 4970–4979.

Tamm, L., Thurig, B., Fliessbach, A., Gottlieb, A.E., Karavani, S., Cohen, Y., 2011. Elicitors and soil management to induce resistance against fungal plant diseases. NJAS-Wagen J. Life Sc. 58, 131–137.

Ubalde, M.C., Brana, V., Sueiro, F., Morel, M.A., Martinez-Rosales, C., Marquez, C., Castro-Sowinski, S., 2012. The versatility of *Delftia* sp isolates as tools for bioremediation and biofertilization technologies. Curr. Microbiol. 64, 597–603.

van Loon, L.C., Geraats, B.P.J., Linthorst, H.J.M., 2006. Ethylene as a modulator of disease resistance in plants. Trends Plant Sci. 11, 184–191.

Vlot, A.C., Dempsey, D.A., Klessig, D.F., 2009. Salicylic Acid, a multifaceted hormone to combat disease. Annu. Rev. Phytopathol. 47, 177–206.

Vyas, P., Gulati, A., 2009. Organic acid production in vitro and plant growth promotion in maize under controlled environment by phosphate-solubilizing fluorescent *Pseudomonas*. BMC Microbiol. 9, 174.