Journal of Ecology

British Ecological Society

Journal of Ecology 2016, **104**, 1566–1575 doi: 10.1111/1365-2745.12626

Systemic enrichment of antifungal traits in the rhizosphere microbiome after pathogen attack

Jan-Hendrik Dudenhöffer[1]*, Stefan Scheu[2] and Alexandre Jousset[3]

[1]Department of Nature Conservation and Landscape Ecology, Institute of Earth and Environmental Sciences, University of Freiburg, Tennenbacher Straße 4, 79106 Freiburg, Germany; [2]Animal Ecology, J. F. Blumenbach Institute of Zoology and Anthropology, Georg August University Göttingen, Berliner Straße 28, 37073 Göttingen, Germany; and [3]Ecology and Biodiversity, Utrecht University, Padualaan 8, 3584CH Utrecht, The Netherlands

Summary

1. Plant-associated microbial communities are crucial for plant growth and play an important role in disease suppression. Community composition and function change upon pathogen attack, yet to date, we do not know whether these changes are a side effect of the infection or actively driven by the plant.

2. Here, we used a split-root approach to test whether barley plants recruit bacteria carrying antifungal traits upon infestation with *Fusarium graminearum*. Split-root systems allow disentangling local infection effects, such as root damage, from systemic, plant-driven effects on microbiome functionality. We assessed the recruitment of fluorescent pseudomonads, a taxon correlated with disease suppression, and of two well-described antifungal genes (*phlD* coding for 2,4-DAPG and *hcnAB* coding for HCN).

3. We show an enrichment of fluorescent pseudomonads, *phlD* and *hcnAB,* upon pathogen infection. This effect was only measurable in the uninfected root compartment. We link these effects to an increased chemotaxis of pseudomonads towards exudates of infected plants.

4. *Synthesis.* We conclude that barley plants selectively recruited bacteria carrying antifungal traits upon pathogen attack and that the pathogen application locally interfered with this process. By disentangling these two effects, we set the base for enhancing strategies unravelling how pathogens and plant hosts jointly shape microbiome functionality.

Key-words: barley, *Fusarium graminearum*, plant–microbe interactions, *Pseudomonas*, recruitment, split-root

Introduction

Plant pathogens cause significant loss of agricultural yield world-wide (Strange & Scott 2005) and are an important determinant of plant community structure and productivity (Packer & Clay 2000; Klironomos 2002; Van der Putten 2003; Petermann *et al.* 2008). Plant-associated microbes form a first line of defence that may complement plant innate immunity. In particular, the rhizosphere microbiome, the microbial community associated with plant roots, is increasingly recognized to shape disease suppression (Berendsen, Pieterse & Bakker 2012; Mendes, Garbeva & Raaijmakers 2013).

The rhizosphere is a place of complex interactions between plants and microbes. Plants invest important resources to secrete root exudates, a set of labile organic compounds able to recruit, feed and manipulate the physiology of a subset of microbes present in the surrounding soil (Bais *et al.* 2006). In return, several of the associated microbes will protect plants against pathogens by producing antibiotics and stimulating plant immunity (Bakker *et al.* 2013).

Plant–microbiome composition varies as a function of plant identity and soil type (Costa *et al.* 2006; Aira *et al.* 2010; Peiffer *et al.* 2013) and even with plant growth stage (Chaparro *et al.* 2013; Yuan *et al.* 2015), indicating that the host plant exerts a structuring influence on the selection of microbial communities from the available species pool. Also plant diseases play an important role and pathogen attack can be associated with alterations in microbiome structure, functionality and activity (Trivedi *et al.* 2012).

These different effects interactively shape microbiome functionality in response to diseases, making it hard to distinguish which changes are actively driven by the host plant (and thus indicate that a plant has the ability to structure its microbiome) and which changes are self-assembly processes driven by changes in the environmental conditions around the roots.

*Correspondence author:
E-mail: jan.dudenhoeffer@nature.uni-freiburg.de

Such passive changes encompass root damage by pathogens, causing leaking of compounds, or an altered exudation in pathogen-challenged plants as a result of a lower photosynthetic activity or a shunting of the nutrient flows towards healthy parts of the root system (Henkes *et al.* 2011). However, there are several cues indicating that plants react to pathogen infection by producing compounds that may attract and stimulate beneficial microbes (Rudrappa *et al.* 2008; Jousset *et al.* 2011). Such plant-mediated processes might even favour specific functions if feedback loops allow plants to reward microbes performing certain tasks, as reported for different plant–microbe symbioses (Phillips *et al.* 2004; Kiers & Denison 2008).

In the present study, we aim to disentangle pathogen- and plant-mediated effects on microbiome functionality. To separate these effects, we set up a split-root system with barley plants growing in a natural soil. Split-root systems involve the separation of the root system into two hermetic compartments, allowing plant–microbe interactions to be tracked independently. In the present case, we added a fungal pathogen to one of the compartments and measured alterations in functional traits of the root-associated communities. At the treatment compartment, the pathogen may alter plant–microbe interactions by preventing exudation or secreting mycotoxins (Notz *et al.* 2002; Henkes *et al.* 2011). In contrast, effects observed on the systemic, non-infected compartments, can be safely attributed to plant-mediated effects (Henkes *et al.* 2011; Jousset *et al.* 2011).

We specifically address whether (i) barley recruits different microbes upon pathogen infection, (ii) whether microbial recruitment is related to an enrichment of antifungal traits (functional genes) and (iii) whether the recruitment process contributes to the structuring of rhizosphere microbial communities. By combining these aspects, we aim to prove the concept that a host plant is able to manipulate its rhizosphere microbiome structure to fit its functionality to the challenges, such as pathogen infection, that the plant is facing.

Materials and methods

STUDY SYSTEM

We used a model system with barley (*Hordeum vulgare* L.) as host plant, *Fusarium graminearum* (Schwabe) as fungal pathogen and fluorescent pseudomonads as potential pathogen antagonists. Barley is the fourth most important cereal crop world-wide. It serves as food for animals and humans as well for malt production (Newton *et al.* 2011), and its general root microbiome structure was recently examined (Bulgarelli *et al.* 2015). One of its major pathogens, the soil-borne fungus *F. graminearum*, is responsible for blight in cereals and a growing concern in agriculture (Goswami & Kistler 2004; Kazan, Gardiner & Manners 2012). In particular, its production of mycotoxins poses a serious threat to animal and human health and makes contaminated products unusable (D'Mello, Placinta & Macdonald 1999).

In an earlier study, Henkes *et al.* (2011) showed that the local infection of barley roots with *F. graminearum* resulted in a reduction in carbon delivery to the infected root parts. An inoculation with fluorescent pseudomonads (*P. protegens* CHA0) counteracted this effect and successfully prevented barley biomass reduction due to the infection. In a further study, Lanoue *et al.* (2010) showed that barley plants changed their exudation profiles after root infection with *F. graminearum*. The plants responded with the synthesis of organic acids exhibiting antifungal activity. Some of these compounds are also known to trigger the production of antifungal metabolites by fluorescent pseudomonads (Jousset *et al.* 2011) and act as chemo-attractants for this bacterial taxon (Oku *et al.* 2014). Based on these evidences, we used this biological system as a model to test our concept that plants may actively manipulate their rhizosphere microbiome in response to an external stressor.

Therefore, we set up two experiments using a split-root approach to disentangle plant- and pathogen mediated effects on microbial communities. The two split-root systems consisted of two separated compartments. One compartment was infected with the pathogen (hereafter the 'treatment compartment') while the other compartment remained pathogen free (hereafter the 'systemic compartment').

SEEDLING PREPARATION

We used barley plants (*Hordeum vulgare* L. cv. 'Barke') grown from commercial seed material (Irnich Inc., Frechen, Germany). Barley seeds were surface-sterilized as described previously (Henkes *et al.* 2011). Briefly, seeds were soaked in H_2SO_4 (62% v/v) for 1 h to remove glumes, washed two times in sterile water and were surface-sterilized in $AgNO_3$ solution (2% w/v) for 20 min. $AgNO_3$ was removed by five cycles of precipitation in 1% NaCl solution and washing in sterile water. Seeds were germinated on 3% water agar at 24 °C in darkness to check for contamination and to obtain roots big enough to be transferred into the split-root systems. One 3-day-old seedling was transferred into each split-root microcosm, and its roots were evenly separated to each of the compartments.

FUNGAL INOCULUM

We used *Fusarium graminearum* (Schwabe) strain FG17 as model pathogen. The fungal cultures were maintained on potato dextrose agar (PDA, Carl Roth GmbH, Essen, Germany) in darkness. Prior to the experimental application, we incubated a cube of actively growing culture of *F. graminearum* in sterile sucrose solution (40 g L^{-1}) for 7 days at room temperature on an orbital shaker at 40 r.p.m. The resulting mycelium suspension was homogenized by placing it through a syringe and then repeatedly centrifuged at 2000 *g* for 2 min, rinsed five times with 0.1× phosphate-buffered solution (PBS) to remove nutrients and finally adjusted to OD_{600} = 0.4 (optical density at 600 nm) in 0.1× PBS. For the chemotaxis experiment, we used a spore suspension of the fungus. Therefore, an actively growing culture was transferred to autoclaved mung bean medium (40 g of mung beans boiled in 1 L water for 20 min) and incubated for 5 days at room temperature on an orbital shaker at 40 r.p.m. The resulting spore suspension was repeatedly centrifuged at 3000 *g* for 5 min, rinsed five times with sterile water and finally adjusted to OD_{600} = 0.1 in sterile water.

SPLIT-ROOT SET-UP

First, we assessed bacterial recruitment in a natural soil (hereafter 'soil experiment'): We grew barley plants in split-root microcosms made of a polypropylene base part, containing the compartment chambers, (width: 78 mm, depth: 6 mm, height: 128 mm) and a

polycarbonate cover plate. Before setting up, the microcosms were autoclaved at 105 °C for 60 min. The compartment chambers were filled with non-sterile soil from the field site of the Jena Experiment (Müller, Scheu & Jousset 2013) and were supplemented with 20% (w/w) sand to avoid soil clumping. Both compartments were irrigated separately with sterile water via fibre–glass capillaries and had apertures on the top to allow for gas exchange (see Fig. S1 in Supporting information). After 10 days growth at 20 °C and 12-h photoperiod under artificial light (120 μmol m^{-2} s^{-1}), the treatment compartment of the split-root microcosms was inoculated with 4 mL of the mycelium suspension, spread evenly on the whole root system. The control plants received the same amount of 0.1× PBS.

Ten days after pathogen addition, the split-root systems were destructively sampled. Rhizosphere soil samples were collected by gently removing the roots with adherent soil and suspended in 30 mL of 0.1× PBS on a horizontal shaker at 60 r.p.m. for 30 min.

We chose the comparatively short growth period of 10 days to allow the challenged plants to respond to the pathogen while preventing too much infection damage which may have caused biases in the interpretation.

MICROBIAL COMMUNITIES

We followed changes in rhizosphere microbial communities at three levels: we assessed total bacterial density as a marker for unspecific changes coming along with the infection, such as nutrient leaking, affecting indiscriminately all microbes. Further, we enumerated the density of fluorescent pseudomonads as model group, which is consistently associated with disease suppression (Mendes *et al.* 2011) and the specific antifungal genes *phlD* and *hcnAB* (as indicators for specific functional gene enrichment). Further, we monitored the diversity of pseudomonads and their community structure to test for selective processes structuring the rhizosphere.

In the second experiment (hereafter 'the chemotaxis experiment'), we tested whether exudates from infected plants are chemo-attractant for bacteria with potential antifungal activity. Therefore, we used a gnotobiotic system where the plants were confronted with the pathogen in otherwise sterile conditions. We built split-root microcosms out of two sterilized 15-mL Falcon tubes (BD Biosciences, Heidelberg, Germany) filled with sterile demineralized water.

Plants were grown for 10 days at 20 °C and 12-h photoperiod after which we inoculated the treatment compartment of half of the plants with 0.5 mL of fungal spore suspension or sterile water, respectively.

After 10 days, the water samples from the root compartments containing the root exudates were filtered through a 0.22 μm filter (Millipore, Merck, Darmstadt, Germany) to remove remaining fungal or root particles and were stored at 4 °C until processing the chemotaxis assay.

CULTURE-BASED ENUMERATION OF BACTERIA – PLATE COUNTS

For the culture-based enumeration of fluorescent pseudomonads and total heterotrophic bacteria, we spread-plated the rhizosphere soil suspensions on Petri dishes with Gould's S1 medium (Gould *et al.* 1985) and R2A medium (Reasoner & Geldreich 1985), respectively. For plating on Gould's S1 medium, we used 50 μL of the 20-fold diluted suspensions and for R2A medium 50 μL of the 100-fold diluted suspensions. Petri dishes were incubated at 28 °C for 48 h in darkness before colonies were counted.

DNA EXTRACTION

DNA extraction was performed in triplicates directly from the soil suspensions, following the protocol of Lueders, Manefield & Friedrich (2004) with slight modifications. Briefly, 600 μL of the soil suspensions was mixed with 150 μL $NaPO_4$ buffer (600 mM), 250 μL TNS solution and 0.7 g of 0.1-mm silica beads. Cells were lysed by beat-beating at 6.5 m s^{-1} for 30 s. The subsequent extraction steps were done according to the protocol. Finally, we eluted the DNA in 30 μL EB buffer. The quality of DNA extracts were checked by electrophoresis on 1.5% agarose gels at 120 V for 30 min. For all further analyses, triplicate samples were pooled.

QUANTITATIVE REAL-TIME PCR

We used quantitative real-time PCR (qPCR) to enumerate total bacteria and specific disease-suppressive bacteria, producing the antifungal metabolites 2,4-diacetylphloroglucinol (2,4-DAPG) and hydrogen cyanide (HCN), which contribute to the suppression of fungal diseases in soil (Voisard *et al.* 1989; Weller *et al.* 2002). All reactions were performed in a final reaction volume of 20 μL containing 2 μL of the template DNA, 10 μL KAPA SYBR FAST qPCR MasterMix Universal (Kapa Biosystems, Wilmington, MA, USA), 24 μg bovine serum albumin (BSA) and 0.24 μM of each of the respective primers. All reactions were run in a Stratagene Mx3005P instrument (Agilent Technologies, Waldbronn, Germany). To enumerate total bacteria, we quantified the copy numbers of 16S rDNA using the primer pair EUB338 and EUB518 (Fierer *et al.* 2005). To enumerate HCN-producing bacteria, we quantified the copy numbers of *hcnAB* using the primer pair PM2 and PM-26R (Svercel, Duffy & Défago 2007), and for 2,4-DAPG-producing bacteria, we quantified the copy numbers of *phlD* using the primer pair BPF2 and BPR4 (McSpadden Gardener *et al.* 2001). As quantification standards, we used a dilution series of already quantified amplification products of the targeted fragments. For reactions targeting 16S and *hcnAB*, the standards were obtained from *P. protegens* strain CHA0 and for *phlD* from *P. fluorescens* strain Q2-87.

Additionally, we performed qPCR targeting *F. graminearum* DNA using the primer pair FG16NF and FG16NR (Nicholson *et al.* 1998) to validate the success of pathogen inoculation and to check for potential cross-contamination. Details of oligonucleotide primers and the cycling conditions are listed in Tables S1 and S2. Microcosms contaminated with *F. graminearum* (presence of the fungus in one of the compartments of control plants or in the systemic compartment of the infected plants, respectively) were omitted from further analyses. This resulted in total of 10 replicated split-root microcosms for the control treatment and 16 replicated microcosms for the infection treatment.

ASSESSMENT OF *PSEUDOMONAS* COMMUNITY STRUCTURE

Pseudomonas community structure was investigated using an established denaturing gel gradient electrophoresis (DGGE) based on a nested PCR amplification of the *Pseudomonas*-specific *gacA* gene (Costa *et al.* 2007). We performed the first PCR using the primer pair gacA-1F (Costa *et al.* 2007) and gacA2 (De Souza, Mazzola & Raaijmakers 2003). PCR was performed in a total reaction volume of 25 μL containing 1 μL of the template DNA, 5 μL KAPA2G buffer A (Kapa Biosystems, Wilmington, MA, USA), 1.25 U KAPA2G Robust DNA polymerase (Kapa Biosystems), 100 μM dNTPs and 0.2 μM of each of the according primer. After purification, the

products were subjected to a second PCR using the primers gacA-1FGC and gacA-2R (Costa *et al.* 2007) in a final reaction volume of 25 μL containing 1 μL of the first PCR product as template, 5 μL KAPA2G buffer A, 1.25 U KAPA2G Robust DNA polymerase, 100 μM dNTPs and 0.2 μM of each of the according primer. Both PCR were performed in a TProffesional Thermal cycler (Analytik Jena, Jena, Germany). Details of oligonucleotide primers and the cycling conditions are listed in the Supporting information (Tables S1 and S2). Electrophoresis was performed on 4 μL of the second PCR product in an 8% acrylamide gel with a denaturing gradient ranging from 20% to 80% denaturants (100% denaturants: 7 M urea and 40% formamide) at 60 °C for 18 h and 140 V in 1× Tris–acetate–EDTA buffer using a Dcode Universal Mutation Detection System (Bio-Rad, Hercules, CA, USA). The gels were stained with 200× SYBR Green (Sigma-Aldrich, Hamburg, Germany). The DGGE fingerprints were digitized to greyscale images and analysed using the QUANTITY ONE software (Bio-Rad).

BACTERIAL CHEMOTAXIS TOWARDS INFECTED AND UNINFECTED PLANTS

We assessed the chemotactic activity of a GFP-tagged *Pseudomonas protegens* CHA0 (Jousset *et al.* 2006) towards the exudates of infected and uninfected plants. We chose this strain as reference fluorescent pseudomonad carrying the functional genes *phlD* and *hcnAB* also targeted in the natural soil experiment. Bacteria were pre-grown in 3 g L^{-1} tryptic soy broth (TSB) at 20 °C for 24 h, centrifuged (5 min at 4500 *g*) and washed 5 times in 0.1× PBS to remove remaining nutrients. Bacterial density was adjusted to OD_{600} = 0.1 in 0.1× PBS. We used 200-μL pipette tips filled with 50 μL of the exudate samples, placed on a multichannel pipette, as chemotaxis capillaries. The filled tips remained on the pipette and were carefully placed into wells of a microtitre plate containing 100 μL of the bacterial suspension. The whole system was incubated for 30 min at 20 °C in darkness to allow the bacteria migrating into the pipette tips. Bacterial concentration in the pipette tips was measured by flow-cytometric enumeration using a BD Accuri C6 flow cytometer (Accuri, Ann Harbor, MI, USA) following the manufacturer's instructions. For both treatments, we set up 10 split-root microcosms as replicates.

STATISTICAL ANALYSES

All analyses were performed in R version 3.1.1 (R Core Team 2014). In the soil experiment, we tested for the effect of the infection treatment on dry shoot biomass using a linear model (LM) with the infection treatment (control vs. pathogen addition) as categorical predictor. To test for differences in root dry biomass, we used a LM with the infection treatment, the compartment side (treatment compartment vs. systemic compartment) and their interaction as categorical predictors.

In the soil experiment, bacterial and gene abundances were calculated as numbers of gene copies and colony-forming units (CFU) per gram soil dry weight, respectively. We calculated *gacA* allelic richness as the number of bands within each gel lane.

Because soil water is essential for bacterial motility, we included the soil water content (percentage soil moisture) as covariate in the models targeting bacterial abundances, gene abundances and *gacA* allelic richness in the soil experiment. The soil water content was not affected by the infection treatment ($F_{1,48}$ = 0.51, P = 0.48), the compartment side ($F_{1,48}$ = 0.06, P = 0.81) nor their interaction ($F_{1,48}$ = 0.60, P = 0.44), indicating that there was no systematic bias.

Bacterial and gene abundances in the soil experiment exhibited a positive, right-skewed distribution with an over proportional increase in variance. Thus, we used generalized linear models (GLM) with gamma-distributed errors and a log-link function (Faraway 2006; Crawley 2007), testing for the effects of the soil water content (linear covariate), the infection treatment (factor with two levels), the compartment side (factor with two levels) and the treatment-compartment side interaction as categorical predictors.

Regarding the analysis of *gacA* allelic richness in the soil experiment, it is noteworthy, that the treatments were represented in equal proportions within a single gel, while the compartment sides were represented on separate gels. Therefore, we tested for the effect of the infection treatment on *gacA* allelic richness separately for the compartment sides using LMs and fitted the gel identity as a block effect before the soil water content and infection treatment. To adjust for the covariate soil water content, we calculated for all above-mentioned response variables their least-square means and standard errors (R-package: lsmeans; Lenth 2015) based on the respective model (GLMs targeting bacterial and gene abundances and LMs targeting *gacA* allelic richness).

For the chemotaxis experiment, we defined chemo-attractant function of root exudates as the density of *P. protegens* CHA0 (cells μL^{-1} exudate) in capillaries filled with the root exudates. We tested for effects of the infection treatment, the compartment side and their interaction as categorical predictors on the chemo-attractant function of root exudates using a GLM with gamma-distributed errors and a log-link function. If the models indicated differing effects of the infection treatment between the compartment sides (infection treatment × compartment side interaction at $P < 0.1$), we additionally tested the pairwise contrasts of the infection treatments within both compartments sides (R-package: multcomp; Hothorn, Bretz & Westfall 2008).

The *gacA* community composition was analysed using non-metric multidimensional scaling (NMDS) based on pairwise Bray–Curtis dissimilarities of the Hellinger-transformed (Legendre & Legendre 1998; Legendre & Gallagher 2001; Ramette 2007) greyscale band intensities. First, ordination was performed separately for each single gel to account for intergel variations resulting from potential differences of the denaturing gradient due to the gel casting procedure. To check for the comparability of the ordination results, we tested for the concordance of the single-gel ordinations within each compartment side using a procrustes rotation test based on 999 permutations. Within the compartment sides, we tested for the significance of the infection treatment on the grouping of the *gacA* ordinations using permutational multivariate analysis of variance (PERMANOVA) based on 999 permutations. Therefore, we constrained the permutations within the single gels to account for gel identity in sense of a statistical block effect (R-package: vegan; Oksanen *et al.* 2013).

Results

Dry shoot biomass of barley plants was not affected by the infection treatment ($F_{1,24}$ = 0.01, P = 0.91). Also dry root biomass was not affected by infection treatment ($F_{1,48}$ = 0.43, P = 0.51), the compartment side ($F_{1,48}$ = 1.78, P = 0.19) nor their interaction ($F_{1,48}$ = 0.04, P = 0.84), indicating that *F. graminearum* did not generate measurable damage during the short incubation time.

The abundance of total heterotrophic bacteria (CFU on R2A medium) was not affected by any predictor (Table 1, Fig. 1a). In contrast, the abundance of fluorescent

Table 1. Results of the generalized linear models (with gamma-distributed errors and a log-link function) targeting the abundances of total heterotrophic bacteria (CFU on R2A medium), fluorescent pseudomonads (CFU on Gould's S1 medium), 16S rDNA, the antifungal genes *hcnAB* and *phlD* in the rhizosphere of barley plants (soil experiment) and the chemo-attractant function of root exudates (chemotaxis experiment). In all models of the soil experiment, the soil water content was fitted as linear covariate before the fixed effects of the infection treatment and the compartment side. Significant effects are highlighted in bold

Response	Predictor	d.f.	Deviance	*F*	*P*
Total heterotrophic bacteria (# CFU $g_{soil\ dry}^{-1}$)	Soil water content	1	0.049	0.538	0.467
	Infection treatment (IT)	1	0.086	0.937	0.338
	Compartment side (CS)	1	0.156	1.698	0.199
	IT × CS	1	0.007	0.072	0.790
	Residuals	47	4.202		
Fluorescent pseudomonads (# CFU $g_{soil\ dry}^{-1}$)	Soil water content	1	5.970	26.818	**< 0.001**
	Infection treatment (IT)	1	1.455	6.538	**< 0.05**
	Compartment side (CS)	1	0.002	0.010	0.920
	IT × CS	1	0.722	3.243	0.078
	Residuals	47	10.226		
16s (# copies $g_{soil\ dry}^{-1}$)	Soil water content	1	0.180	3.389	0.072
	Infection treatment (IT)	1	0.104	1.969	0.167
	Compartment side (CS)	1	0.001	0.017	0.897
	IT × CS	1	0.002	0.044	0.836
	Residuals	47	2.471		
phlD (# copies $g_{soil\ dry}^{-1}$)	Soil water content	1	9.503	14.205	**< 0.001**
	Infection treatment	1	0.891	1.332	0.254
	Compartment side	1	0.949	1.418	0.240
	IT × CS	1	3.459	5.171	**< 0.05**
	Residuals	47	32.337		
hcnAB (# copies $g_{soil\ dry}^{-1}$)	Soil water content	1	6.264	18.002	**< 0.001**
	Infection treatment (IT)	1	0.273	0.783	0.381
	Compartment side (CS)	1	0.073	0.210	0.649
	IT × CS	1	2.522	7.247	**< 0.01**
	Residuals	47	18.352		
Chemo-attractant function (cells μL^{-1} exudate)	Infection treatment (IT)	1	1.529	4.457	**< 0.05**
	Compartment side (CS)	1	0.260	0.759	0.389
	IT × CS	1	0.109	0.317	0.577
	Residuals	36	12.350		

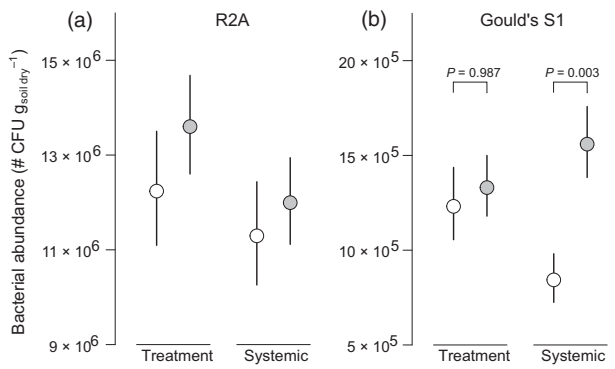

Fig. 1. Abundance (least-square means and standard errors) of (a) total heterotrophic bacteria (CFU on R2A medium) and (b) fluorescent pseudomonads (CFU on Gould's S1 medium) in the rhizosphere of uninfected plants (white circles, left) and infected plants (grey circles, right) of the soil experiment. Treatment compartments are paired to the left and the systemic compartments are paired to the right. For significant infection treatment x compartment side interactions ($P < 0.1$) additional *P*-values are given for the pairwise contrasts of the infection treatment within each compartment side..

pseudomonads (CFU on Gould's S1 medium) was higher in the rhizosphere of infected plants than of uninfected plants at the systemic compartment (Table 1, Fig. 1b). Further, the abundance of fluorescent pseudomonads was positively correlated with the soil water content (Table 1, Fig. 2a).

Similar to the abundance of total heterotrophic bacteria, there were no effects of any predictor on the abundance of 16S rDNA gene copy numbers (Table 1, Fig. 3a). However, the abundances of the two assessed antifungal genes (*phlD* and *hcnAB*) were positively correlated with the soil water content (Table 1, Fig. 2b,c). The effect of the infection treatment on antifungal gene abundance depended on the compartment side (Table 1) and their abundances were higher in the rhizosphere of infected plants than of uninfected plants at the systemic compartments (Fig. 3a,b). In the treatment compartment, *gacA* allelic richness was not significantly affected by the infection treatment (Table 2). However, in the systemic compartment, *gacA* allelic richness was higher in the rhizosphere of infected plants than of uninfected plants (Table 2, Fig. 4a).

Procrustes rotation test revealed that the single-gel ordinations of the *gacA* DGGE profiles were congruent in the systemic compartments ($P = 0.017$), indicating a coherent structuring force on the communities. Further, community composition was well discriminated by the infection treatment in the systemic compartment (Table 2, Fig. 4b). The single-

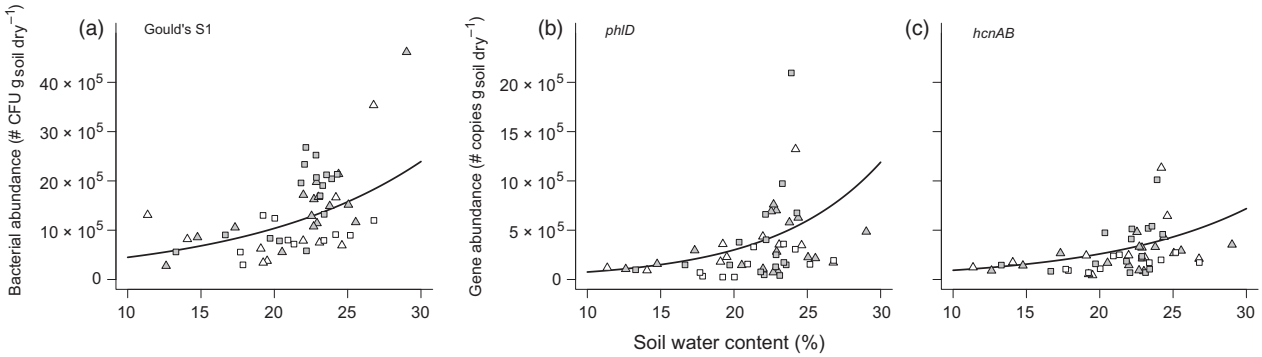

Fig. 2. Effect of the soil water content on the abundances of (a) fluorescent pseudomonads (CFU on Gould's S1 medium) and the functional genes (b) *phlD* and (c) *hcnAB* in the rhizosphere of uninfected (white) and infected (grey) plants at the treatment compartments (triangles) and the systemic compartments (squares) of the soil experiment. Regression fits refer to the respective generalized linear model (with gamma distributed errors and a log-link function).

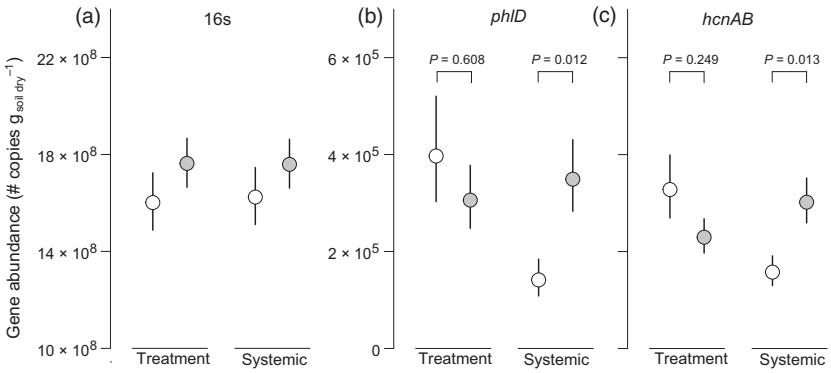

Fig. 3. Abundance (least-square means and standard errors) of (a) 16s rDNA and the functional genes (b) *phlD* and (c) *hcnAB* in the rhizosphere of uninfected plants (white circles, left) and infected plants (grey circles, right) of the soil experiment. Treatment compartments are paired to the left and the systemic compartments are paired to the right. For significant infection treatment x compartment side interactions ($P < 0.1$) additional *P*-values are given for the pairwise contrasts of the infection treatment within each compartment side.

Table 2. Results of the linear models (LM) targeting *gacA* allelic richness and the permutational multivariate analysis of variance (PERMANOVA) targeting the differences in the *gacA* community compositions. All analyses were performed separately for each compartment side. The gel identity was fitted before the soil water content and the infection treatment in the LMs. Permutations in the PERMANOVAS were constrained within the single gels. Significant effects are highlighted in bold

Response	Compartment side	Predictor	d.f.	SS	*F*	*P*
gacA richness (# bands)	Treatment	Gel identity	1	30.154	4.560	< **0.05**
		Soil water content	1	0.529	0.080	0.778
		Infection treatment	1	4.307	0.651	0.428
		Residuals	22	145.472		
	Systemic	Gel identity	1	5.538	2.632	0.119
		Soil water content	1	6.135	2.916	0.102
		Infection treatment	1	42.034	19.976	< **0.001**
		Residuals	22	46.293		
gacA composition (NMDS)	Treatment	Infection treatment	1	0.023	0.377	0.767
		Residuals	24	1.472		
	Systemic	Infection treatment	1	0.185	2.018	< **0.001**
		Residuals	24	2.197		

gel ordinations in the treatment compartment were not congruent ($P = 0.15$), and community composition was not separated regarding the infection treatment (Table 2, Fig. 4b).

In the chemotaxis experiment, *P. protegens* CHA0 was more attracted by the root exudates of infected plants than by the root exudates of the control plants. This increase was independent of the compartment side (Table 1, Fig. 5).

Discussion

The results of our study show an enrichment of the rhizosphere microbiome with potentially antifungal microbes for barley plants challenged with *F. graminearum*. By combining a split-root approach with different levels of resolution (zooming from all bacteria into one representative taxonomic group

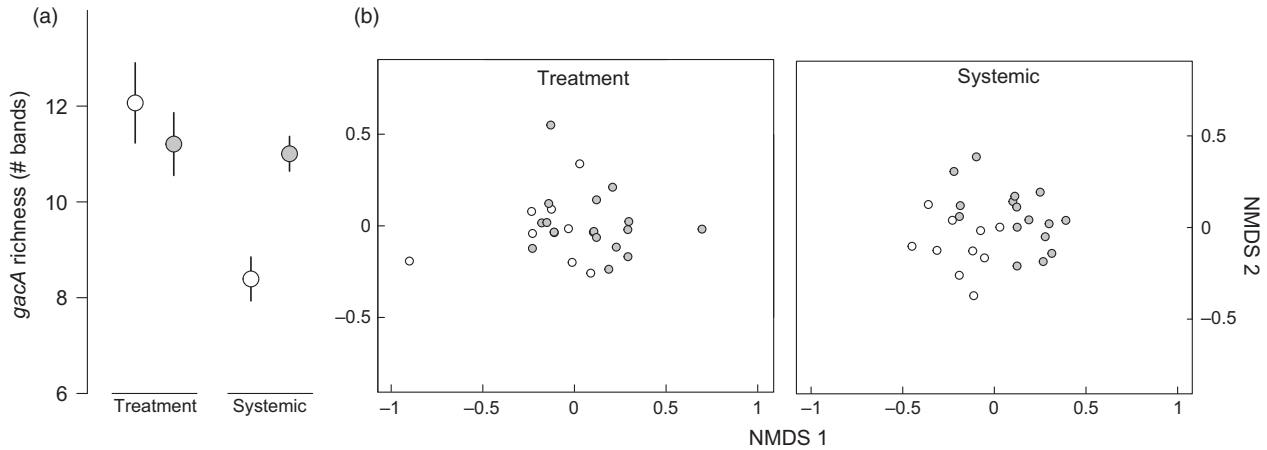

Fig. 4. *gacA* allelic richness (a) (least-square means and standard errors) and *gacA* community composition (based on NMDS ordination) (b) in the rhizosphere of uninfected plants (white circles, left) and infected plants (grey circles, right) of the soil experiment. In panel (a) treatment compartments are paired to the left and the systemic compartments are paired to the right. In panel (b) each point represents one single plant. For both compartment sides, the results from the single gel ordinations are superimposed to aid visualization.

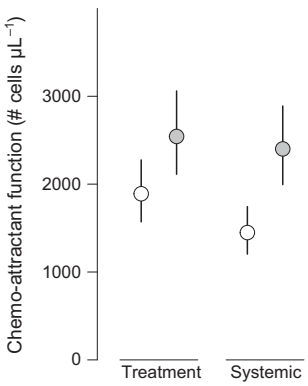

Fig. 5. Chemo-attractant function (least-square means and standard errors) of *P. protegens* towards the root exudates of uninfected plants (white circles, left) and infected plants (grey circles, right) of the chemotaxis experiment. Treatment compartments are paired to the left and the systemic compartments are paired to the right.

and two representative functional genes), we could disentangle the effects of plant-mediated microbiome structuring from perturbations of the infection treatment.

Our results provide evidence for the concept that plants are able to actively recruit specific bacterial groups upon pathogen infection. Fluorescent pseudomonad abundance increased with infection. These bacteria are good colonizers of plant roots (Loper *et al.* 2012), and we propose that their enrichment in the rhizosphere of infected plants may be explained by conserved characteristics such as chemotactic activity towards root exudates. The fact that fluorescent pseudomonads are repeatedly associated with disease suppression (Weller 2007; Mendes *et al.* 2011) makes it plausible that a co-evolutionary process between plants and this bacterial group occurred, in which bacteria evolved to respond to plant alarm cues.

However, we can only speculate about the underlying mechanism. In contrast to symbioses with rhizobia or mycorrhiza, plants have very few possibilities to select for partners providing a particular function such as disease protection (Kiers & Denison 2008). We propose that feedback loops, such as the enhanced exudation in presence of 2,4-DAPG (Phillips *et al.* 2004), may provide a reward mechanism that may have promoted the recruitment of bacteria harbouring antifungal traits.

Bacterial recruitment can be the result of a specific attraction by exudates or by a higher growth on roots. Chemotaxis appears here as a significant driver. Exudates from infected plants were more attractive than exudates of uninfected plants for *P. protegens* CHA0, a model strain harbouring the genes needed for the production of both 2,4-DAPG and HCN, the two genes that were systemically enriched in the rhizosphere of infected plants. These results are in line with a past study on *Bacillus*, showing that plants challenged by the foliar pathogen *Pseudomonas syringae* recruited one specific *Bacillus* strain by secreting more malic acid, to which the studied *Bacillus* strain showed a strong chemotactic response (Rudrappa *et al.* 2008). We propose that a similar effect occurred in the soil experiment with fluorescent pseudomonads. The chemotaxis hypothesis is further backed by the importance of the soil water content for bacterial recruitment. High soil moisture promotes the motility of free-living bacteria (Wong & Griffin 1976) as well as the diffusion of soluble nutrients and chemo-attractant compounds in the soil matrix (Raynaud 2010). The positive effect of the soil water content on the abundance of fluorescent pseudomonads and antifungal genes indicates that the recruitment process is driven by increased migration of motile bacteria towards the rhizosphere of the plants. Fluorescent pseudomonads are highly motile bacteria, which is an important trait related to their rhizosphere competence (Turnbull *et al.* 2001; De Weert *et al.* 2002; Martínez-Granero, Rivilla & Martín 2006). The hypothesis of plant triggered migration of disease-suppressive bacteria is also supported by the chemotaxis experiment, documenting that sterile root exudates from infected plants were more attractive to *P. protegens* than those from control plants. Root exudates serve the plant to communicate with surrounding

microbes and their composition can shift in presence of pathogens. For instance, organic acids exert an attracting cue for the chemotactic activity of fluorescent pseudomonads (Oku *et al.* 2014). Plants can rapidly change their exudation profile in response to pathogen infection and initiate the synthesis of defensive organic acids to suppress pathogens (Lanoue *et al.* 2010). Some of these compounds also stimulate the expression of antifungal genes (Jousset *et al.* 2011).

Alternatively, an increased water potential can rapidly lead to an increased bacterial biomass (Lund & Goksøyr 1980; Kieft, Soroker & Firestone 1987). In both scenarios, we propose that sufficiently high soil moisture is essential for the plant to recruit beneficial microbes out of the existing microbial species pool.

Pseudomonad richness, measured as *gacA* allelic richness, was higher in the rhizosphere of infected plants than of uninfected plants. This fits with an enhanced chemo-attractant function of the root exudates of infected plants. For instance, infected plants may have recruited bacteria from more distant soil regions, which would allow accessing a broader microbial species pool. This higher diversity may be crucial for efficient disease suppression as it may enhance the production of antifungal metabolites (Jousset *et al.* 2014). The distinctiveness of the bacterial community compositions between the rhizosphere of infected plants and uninfected plants at the systemic compartments further suggests that this selective plant-driven recruitment functions as a structuring force for the assembly of rhizosphere bacteria.

CAVEATS

We observed enrichments of specific bacteria due to the infection treatment in the systemic compartment only. This leads us to propose that the inoculum application may locally interfere with plant-driven effects as observed at the systemic compartments. The addition of PBS to the treatment compartment may have affected microbial communities via an addition of phosphorus or a mechanical perturbation of the root system. Even if we kept perturbation as low as possible by using highly diluted PBS and dripping it slowly onto the roots, it may account for some effects such as the higher bacterial abundances in the treatment compartment than the systemic compartment. Also effects of the fungal inoculum itself, for example an antagonistic (Notz *et al.* 2002) or attracting potential of fungal metabolites (De Weert *et al.* 2004), may mask plant-driven processes. Such concomitant effects illustrate how important it is to physically separate the site of experimental manipulation from the site of measurements especially in the context of systemic plant-driven processes.

This study focused on one specific plant–pathogen system and on three very simplified bacterial groups (all bacteria, one specific taxon and two functional genes). Also the plants were grown for a short time in small microcosms under the controlled conditions of a climate chamber. The potential differences to other plant–pathogen systems and the impact of environmental conditions or plant age remain a subject for further investigation, which would give valuable insights into the generality and limitations of the process observed in this study. Nevertheless, this simplification allowed us to conceptually demonstrate a new dimension in plant–microbiome interactions, disentangling effects of a host plant and a pathogen on the composition and functionality of microbial communities. However, this approach also brings a few caveats we would like to address. First, we would like to emphasize that the tested groups are examples. Microbial communities are very diverse, both phylogenetically and functionally. Further, we did not attempt to analyse exudate composition, as they would have in the present context added few to the observed net effects on bacteria. Rather, we see our approach as a conceptual base that can be combined with metabolomics or metagenomics to disentangle the roles of plant and pathogen on the assembly of the rhizosphere microbiome. Further, we did not assess the antifungal potential of root-associated bacteria. We propose that future studies assessing soil suppressiveness in the systemic compartment after several growth cycles may greatly improve our understanding of the role of the plants in building up soil suppressiveness as observed in take-all decline (Weller *et al.* 2002).

Conclusion

We demonstrated that barley plants actively manipulate their rhizosphere community to favour a specific function, such as here antifungal traits, in response to pathogen attack. This extends the finding from Jousset *et al.* (2011) showing a stimulation of antifungal gene expression in bacteria living on pathogen infested plants to the recruitment and enrichment of disease-suppressive bacteria in a non-sterile soil system. The specificity of the recruitment indicates an active selection process by the plant, and its restriction to non-infected parts of the root systems suggests that pathogen presence or infection-linked damages to the plant may reduce the ability of plants to select for beneficial microbes. The identification of the ability of plants to recruit beneficial microbes to counteract pathogens remains a challenge for plant breeding (Haney *et al.* 2015). Our study provides a new strategy to assess a plant`s potential to recruit disease-suppressive bacteria by removing the noise caused by pathogen infection.

Acknowledgements

We thank Petr Karlovsky (Department of Molecular Phytopathology and Mycotoxin Research, University of Göttingen, Germany) for providing the fungal cultures and the primers used for fungal detection in the soil experiment.

Data accessibility

Data associated with this study are deposited in the Dryad Digital Repository http://dx.doi.org/10.5061/dryad.83np7 (Dudenhöffer, Scheu & Jousset 2016).

References

Aira, M., Gómez-Brandón, M., Lazcano, C., Bååth, E. & Domínguez, J. (2010) Plant genotype strongly modifies the structure and growth of maize rhizosphere microbial communities. *Soil Biology and Biochemistry*, **42**, 2276–2281.

Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S. & Vivanco, J.M. (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology*, **57**, 233–266.

Bakker, P.A., Doornbos, R.F., Zamioudis, C., Berendsen, R.L. & Pieterse, C.M. (2013) Induced systemic resistance and the rhizosphere microbiome. *The Plant Pathology Journal*, **29**, 136–143.

Berendsen, R.L., Pieterse, C.M. & Bakker, P.A. (2012) The rhizosphere microbiome and plant health. *Trends in Plant Science*, **17**, 478–486.

Bulgarelli, D., Garrido-Oter, R., Münch, P.C., Weiman, A., Dröge, J., Pan, Y., McHardy, A.C. & Schulze-Lefert, P. (2015) Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell Host & Microbe*, **17**, 392–403.

Chaparro, J.M., Badri, D.V., Bakker, M.G., Sugiyama, A., Manter, D.K. & Vivanco, J.M. (2013) Root exudation of phytochemicals in Arabidopsis follows specific patterns that are developmentally programmed and correlate with soil microbial functions. *PLoS One*, **8**, e55731.

Costa, R.G., Götz, M., Mrotzek, N., Lottmann, J., Berg, G. & Smalla, K. (2006) Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. *FEMS Microbiology Ecology*, **56**, 236–249.

Costa, R.G., Newton, C.M., Kroegerrecklenfort, E., Opelt, K., Berg, G. & Smalla, K. (2007) *Pseudomonas* community structure and antagonistic potential in the rhizosphere: insights gained by combining phylogenetic and functional gene-based analyses. *Environmental Microbiology*, **9**, 2260–2273.

Crawley, M.J. (2007) *The R Book*. Wiley, Chichester, UK.

De Souza, J.T., Mazzola, M. & Raaijmakers, J.M. (2003) Conservation of the response regulator gene *gacA* in *Pseudomonas* species. *Environmental Microbiology*, **5**, 1328–1340.

De Weert, S., Vermeiren, H., Mulders, I.H., Kuiper, I., Hendrickx, N., Bloemberg, G.V., Vanderleyden, J., de Mot, R. & Lugtenberg, B.J. (2002) Flagella-driven chemotaxis towards exudate components is an important trait for tomato root colonization by *Pseudomonas fluorescens*. *Molecular Plant-Microbe Interactions*, **15**, 1173–1180.

De Weert, S., Kuiper, I., Lagendijk, E.L., Lamers, G.E. & Lugtenberg, B.J. (2004) Role of chemotaxis toward fusaric acid in colonization of hyphae of *Fusarium oxysporum* f. sp. *radicis-lycopersici* by *Pseudomonas fluorescens* WCS365. *Molecular Plant-Microbe Interactions*, **17**, 1185–1191.

D'Mello, J., Placinta, C.M. & Macdonald, A. (1999) Fusarium mycotoxins: a review of global implications for animal health, welfare and productivity. *Animal Feed Science and Technology*, **80**, 183–205.

Dudenhöffer, J.-H., Scheu, S. & Jousset, A. (2016) Data from: Systemic enrichment of antifungal traits in the rhizosphere microbiome after pathogen attack. *Dryad Digital Repository*, http://dx.doi.org/10.5061/dryad.83np7.

Faraway, J.J. (2006) *Extending the Linear Model With R. Generalized Linear, Mixed Effects and Nonparametric Regression Models*. Chapman & Hall/CRC, Boca Raton, FL, USA.

Fierer, N., Jackson, J.A., Vilgalys, R. & Jackson, R.B. (2005) Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Applied and Environmental Microbiology*, **71**, 4117–4120.

Goswami, R.S. & Kistler, H.C. (2004) Heading for disaster: *Fusarium graminearum* on cereal crops. *Molecular Plant Pathology*, **5**, 515–525.

Gould, W.D., Hagedorn, C., Bardinelli, T.R. & Zablotowicz, R.M. (1985) New selective media for enumeration and recovery of fluorescent pseudomonads from various habitats. *Applied and Environmental Microbiology*, **49**, 28–32.

Haney, C.H., Samuel, B.S., Bush, J. & Ausubel, F.M. (2015) Associations with rhizosphere bacteria can confer an adaptive advantage to plants. *Nature Plants*, **1**, 15051.

Henkes, G.J., Jousset, A., Bonkowski, M., Thorpe, M.R., Scheu, S., Lanoue, A., Schurr, U. & Röse, U.S. (2011) *Pseudomonas fluorescens* CHA0 maintains carbon delivery to *Fusarium graminearum*-infected roots and prevents reduction in biomass of barley shoots through systemic interactions. *Journal of Experimental Botany*, **62**, 4337–4344.

Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous inference in general parametric models. *Biometrical Journal*, **50**, 346–363.

Jousset, A., Lara, E., Wall, L.G. & Valverde, C. (2006) Secondary metabolites help biocontrol strain *Pseudomonas fluorescens* CHA0 to escape protozoan grazing. *Applied and Environmental Microbiology*, **72**, 7083–7090.

Jousset, A., Rochat, L., Lanoue, A., Bonkowski, M., Keel, C. & Scheu, S. (2011) Plants respond to pathogen infection by enhancing the antifungal gene expression of root-associated bacteria. *Molecular Plant-Microbe Interactions*, **24**, 352–358.

Jousset, A., Becker, J., Chatterjee, S., Karlovsky, P., Scheu, S. & Eisenhauer, N. (2014) Biodiversity and species identity shape the antifungal activity of bacterial communities. *Ecology*, **95**, 1184–1190.

Kazan, K., Gardiner, D.M. & Manners, J.M. (2012) On the trail of a cereal killer: recent advances in *Fusarium graminearum* pathogenomics and host resistance. *Molecular Plant Pathology*, **13**, 399–413.

Kieft, T.L., Soroker, E. & Firestone, M.K. (1987) Microbial biomass response to a rapid increase in water potential when dry soil is wetted. *Soil Biology and Biochemistry*, **19**, 119–126.

Kiers, E.T. & Denison, R.F. (2008) Sanctions, cooperation, and the stability of plant-rhizosphere mutualisms. *Annual Review of Ecology, Evolution, and Systematics*, **39**, 215–236.

Klironomos, J.N. (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature*, **417**, 67–70.

Lanoue, A., Burlat, V., Henkes, G.J., Koch, I., Schurr, U. & Röse, U.S. (2010) De novo biosynthesis of defense root exudates in response to *Fusarium* attack in barley. *New Phytologist*, **185**, 577–588.

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

Legendre, P. & Legendre, L. (1998) *Numerical Ecology*. Elsevier, Amsterdam, the Netherlands.

Lenth, R.V. (2015) *lsmeans: Least-Squares Means*. R-package version 2.20-2. Available at: http://CRAN.R-project.org/package=lsmeans.

Loper, J.E., Hassan, K.A., Mavrodi, D.V., Davis, E.W., Lim, C.K., Shaffer, B.T. *et al.* (2012) Comparative genomics of plant-associated *Pseudomonas* spp.: insights into diversity and inheritance of traits involved in multitrophic interactions. *PLoS Genetics*, **8**, e1002784.

Lueders, T., Manefield, M. & Friedrich, M.W. (2004) Enhanced sensitivity of DNA- and rRNA-based stable isotope probing by fractionation and quantitative analysis of isopycnic centrifugation gradients. *Environmental Microbiology*, **6**, 73–78.

Lund, V. & Goksøyr, J. (1980) Effects of water fluctuations on microbial mass and activity in soil. *Microbial Ecology*, **6**, 115–123.

Martínez-Granero, F., Rivilla, R. & Martín, M. (2006) Rhizosphere selection of highly motile phenotypic variants of *Pseudomonas fluorescens* with enhanced competitive colonization ability. *Applied and Environmental Microbiology*, **72**, 3429–3434.

McSpadden Gardener, B.B., Mavrodi, D.V., Thomashow, L.S. & Weller, D.M. (2001) A rapid polymerase chain reaction-based assay characterizing rhizosphere populations of 2,4-diacetylphloroglucinol-producing bacteria. *Phytopathology*, **91**, 44–54.

Mendes, R., Kruijt, M., de Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J.H., Piceno, Y.M., DeSantis, T.Z., Andersen, G.L., Bakker, P.A. & Raaijmakers, J.M. (2011) Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science*, **332**, 1097–1100.

Mendes, R., Garbeva, P. & Raaijmakers, J.M. (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiology Reviews*, **37**, 634–663.

Müller, M.S., Scheu, S. & Jousset, A. (2013) Protozoa drive the dynamics of culturable biocontrol bacterial communities. *PLoS One*, **8**, e66200.

Newton, A.C., Flavell, A.J., George, T.S., Leat, P., Mullholland, B., Ramsay, L. *et al.* (2011) Crops that feed the world 4. Barley: a resilient crop? Strengths and weaknesses in the context of food security. *Food Security*, **3**, 141–178.

Nicholson, P., Simpson, D., Weston, G., Rezanoor, H., Lees, A., Parry, D. & Joyce, D. (1998) Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. *Physiological and Molecular Plant Pathology*, **53**, 17–37.

Notz, R., Maurhofer, M., Dubach, H., Haas, D. & Defago, G. (2002) Fusaric acid-producing strains of *Fusarium oxysporum* alter 2,4-Diacetylphloroglucinol biosynthetic gene expression in *Pseudomonas fluorescens* CHA0 in vitro and in the rhizosphere of wheat. *Applied and Environmental Microbiology*, **68**, 2229–2235.

Oksanen, J., Blanchet, G.F., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H. & Wagner, H. (2013) *vegan: Community Ecology Package*. R-package version 2.0-10. Available at: http://CRAN.R-project.org/package=vegan.

Oku, S., Komatsu, A., Nakashimada, Y., Tajima, T. & Kato, J. (2014) Identification of *Pseudomonas fluorescens* chemotaxis sensory proteins for malate, succinate, and fumarate, and their involvement in root colonization. *Microbes and Environments*, **29**, 413–419.

Packer, A. & Clay, K. (2000) Soil pathogens and spatial patterns of seedling mortality in a temperate tree. *Nature*, **404**, 278–281.

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. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 6548–6553.

Petermann, J.S., Fergus, A.J.F., Turnbull, L.A. & Schmid, B. (2008) Janzen–Connell effects are widespread and strong enough to maintain diversity in grasslands. *Ecology*, **89**, 2399–2406.
Phillips, D.A., Fox, T.C., King, M.D., Bhuvaneswari, T.V. & Teuber, L.R. (2004) Microbial products trigger amino acid exudation from plant roots. *Plant Physiology*, **136**, 2887–2894.
R Core Team (2014) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org.
Ramette, A. (2007) Multivariate analyses in microbial ecology. *FEMS Microbiology Ecology*, **62**, 142–160.
Raynaud, X. (2010) Soil properties are key determinants for the development of exudate gradients in a rhizosphere simulation model. *Soil Biology and Biochemistry*, **42**, 210–219.
Reasoner, D.J. & Geldreich, E.E. (1985) A new medium for the enumeration and subculture of bacteria from potable water. *Applied and Environmental Microbiology*, **49**, 1–7.
Rudrappa, T., Czymmek, K.J., Paré, P.W. & Bais, H.P. (2008) Root-secreted malic acid recruits beneficial soil bacteria. *Plant Physiology*, **148**, 1547–1556.
Strange, R.N. & Scott, P.R. (2005) Plant disease: a threat to global food security. *Annual Review of Phytopathology*, **43**, 83–116.
Svercel, M., Duffy, B. & Défago, G. (2007) PCR amplification of hydrogen cyanide biosynthetic locus *hcnAB* in *Pseudomonas* spp. *Journal of Microbiological Methods*, **70**, 209–213.
Trivedi, P., He, Z., Nostrand, Van., Joy, D., Albrigo, G., Zhou, J. & Wang, N. (2012) Huanglongbing alters the structure and functional diversity of microbial communities associated with citrus rhizosphere. *The ISME Journal*, **6**, 363–383.
Turnbull, G.A., Morgan, J.W., Whipps, J.M. & Saunders, J.R. (2001) The role of bacterial motility in the survival and spread of *Pseudomonas fluorescens* in soil and in the attachment and colonisation of wheat roots. *FEMS Microbiology Ecology*, **36**, 21–31.
Van der Putten, W.H. (2003) Plant defense belowground and spatiotemporal processes in natural vegetation. *Ecology*, **84**, 2269–2280.
Voisard, C., Keel, C., Haas, H. & Défago, G. (1989) Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. *EMBO Journal*, **8**, 351–358.
Weller, D.M. (2007) *Pseudomonas* biocontrol agents of soilborne pathogens: looking back over 30 years. *Phytopathology*, **97**, 250–256.
Weller, D.M., Raaijmakers, J.M., Gardener, B.B. & Thomashow, L.S. (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annual Review of Phytopathology*, **40**, 309–348.
Wong, P. & Griffin, D.M. (1976) Bacterial movement at high matric potentials – I. In artificial and natural soils. *Soil Biology and Biochemistry*, **8**, 215–218.
Yuan, J., Chaparro, J.M., Manter, D.K., Zhang, R., Vivanco, J.M. & Shen, Q. (2015) Roots from distinct plant developmental stages are capable of rapidly selecting their own microbiome without the influence of environmental and soil edaphic factors. *Soil Biology and Biochemistry*, **89**, 206–209.

Received 9 December 2015; accepted 8 June 2016
Handling Editor: James Cahill

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Specifications of the split-root microcosms used in the soil experiment (base part).

Figure S2. Set-up of the split-root microcosms used in the soil experiment.

Table S1. Specifications of oligonucleotide primers.

Table S2. PCR cycling conditions.