



## Breeding for soil-borne pathogen resistance impacts active rhizosphere microbiome of common bean

Lucas William Mendes<sup>1,2</sup> · Rodrigo Mendes<sup>3</sup> · Jos M. Raaijmakers<sup>2,4</sup> · Siu Mui Tsai<sup>1</sup>

Received: 27 April 2018 / Revised: 13 June 2018 / Accepted: 15 June 2018  
© International Society for Microbial Ecology 2018

### Abstract

Over the past century, plant breeding programs have substantially improved plant growth and health, but have not yet considered the potential effects on the plant microbiome. Here, we conducted metatranscriptome analysis to determine if and how breeding for resistance of common bean against the root pathogen *Fusarium oxysporum* (*Fox*) affected gene expression in the rhizobacterial community. Our data revealed that the microbiome of the *Fox*-resistant cultivar presented a significantly higher expression of genes associated with nutrient metabolism, motility, chemotaxis, and the biosynthesis of the antifungal compounds phenazine and colicin V. Network analysis further revealed a more complex community for *Fox*-resistant cultivar and indicated *Paenibacillus* as a keystone genus in the rhizosphere microbiome. We suggest that resistance breeding in common bean has unintentionally co-selected for plant traits that strengthen the rhizosphere microbiome network structure and enrich for specific beneficial bacterial genera that express antifungal traits involved in plant protection against infections by root pathogens.

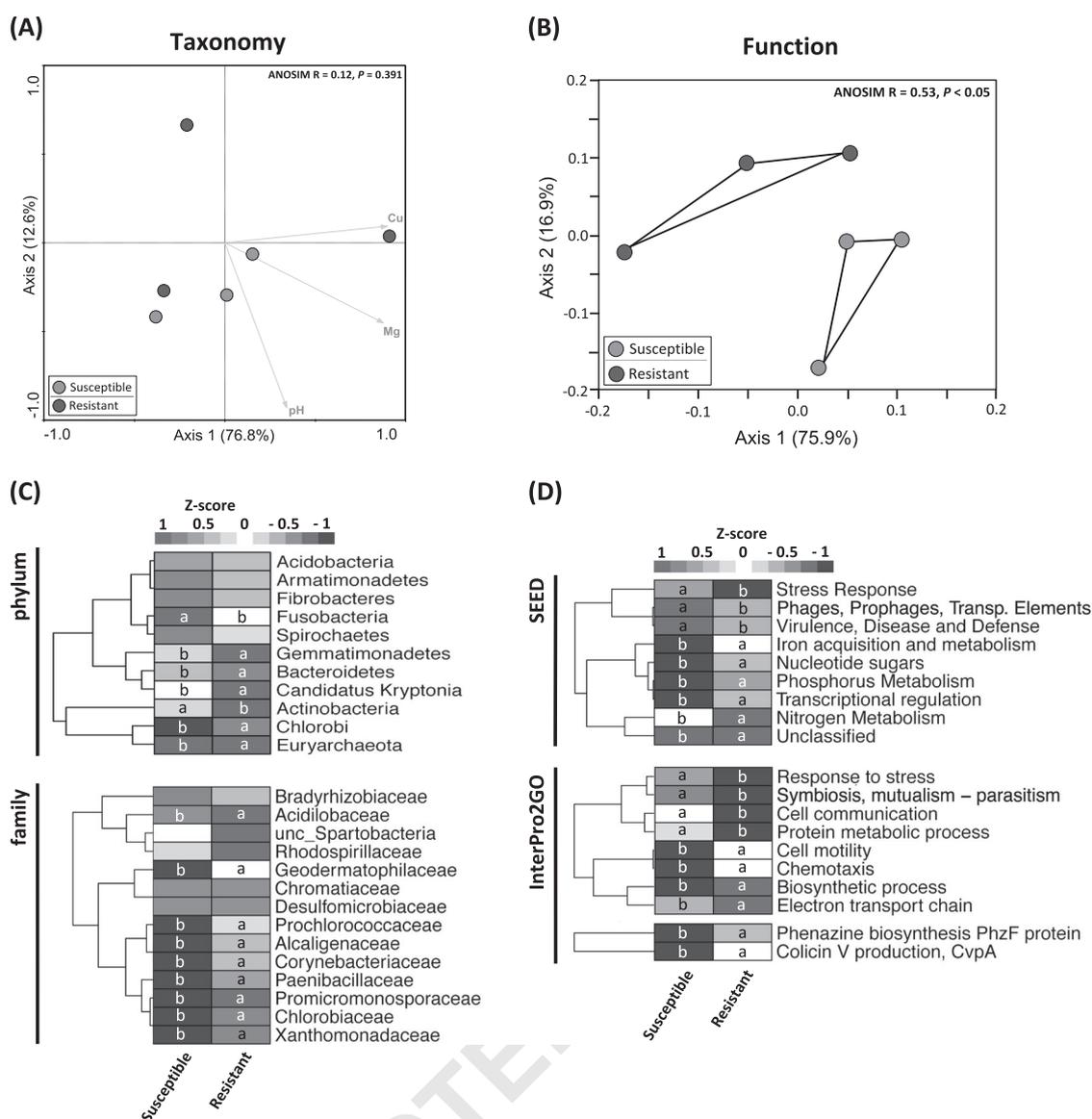
The rhizosphere microbiome significantly impacts plant growth, development and resistance against soil-borne pathogens [1]. Plants shape their rhizosphere microbiome through exudates that stimulate or repress the abundance of specific microbial groups [2]. In return, rhizosphere microbes provide a range of ecosystem services for the plant, such as nutrient acquisition [3], abiotic stress tolerance [4], and protection against pathogens either directly via antagonism or indirectly via induction of systemic resistance [5, 6]. Recent studies further indicated that plant breeding for disease resistance has affected microbiome

assembly in the rhizosphere [7, 8]. Considering that the rhizosphere microbiome provides a first line of defense for plants against pathogen invasion, we investigated if and how breeding of common bean (*Phaseolus vulgaris*) to the soil-borne pathogen *Fusarium oxysporum* f. sp. *phaseoli* (*Fox*) affected gene expression in the rhizosphere microbiome. In our previous study [7], using 16S rRNA and metagenome sequencing, we showed that beneficial bacterial groups such as *Pseudomonas* and *Bacillus*, and genes involved in the biosynthesis of phenazines and rhamnolipids were more abundant in the rhizosphere of *Fox*-resistant bean cultivar. Whether these bacterial genera are active and if the enriched genes are actually expressed in the rhizosphere of the *Fox*-resistant cultivar remains unclear. To further investigate the biological relevance of these DNA-based descriptive analyses, we set out a metatranscriptome analysis (RNA-based) to test if these and other putative bacterial genes are differentially expressed in the rhizosphere of the *Fox*-resistant cultivar as compared to the *Fox*-susceptible. Here, both cultivars were grown under the same controlled conditions in a greenhouse experiment and metatranscriptome analysis was used to assess the active microbial taxa and functions in the rhizosphere of two common beans with contrasting levels of resistance to the soil-borne pathogen.

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1038/s41396-018-0234-6>) contains supplementary material, which is available to authorized users.

✉ Lucas William Mendes  
lucaswmendes@gmail.com

- <sup>1</sup> Cell and Molecular Biology Laboratory, Center for Nuclear Energy in Agriculture CENA University of Sao Paulo USP, Piracicaba, SP 13416-000, Brazil
- <sup>2</sup> Departament of Microbial Ecology, Netherlands Institute of Ecology NIOO-KNAW, Wageningen 6708 PB, The Netherlands
- <sup>3</sup> Embrapa Meio Ambiente, Jaguariuna 18020-000, Brazil
- <sup>4</sup> Institute of Biology, Leiden University, Leiden, The Netherlands

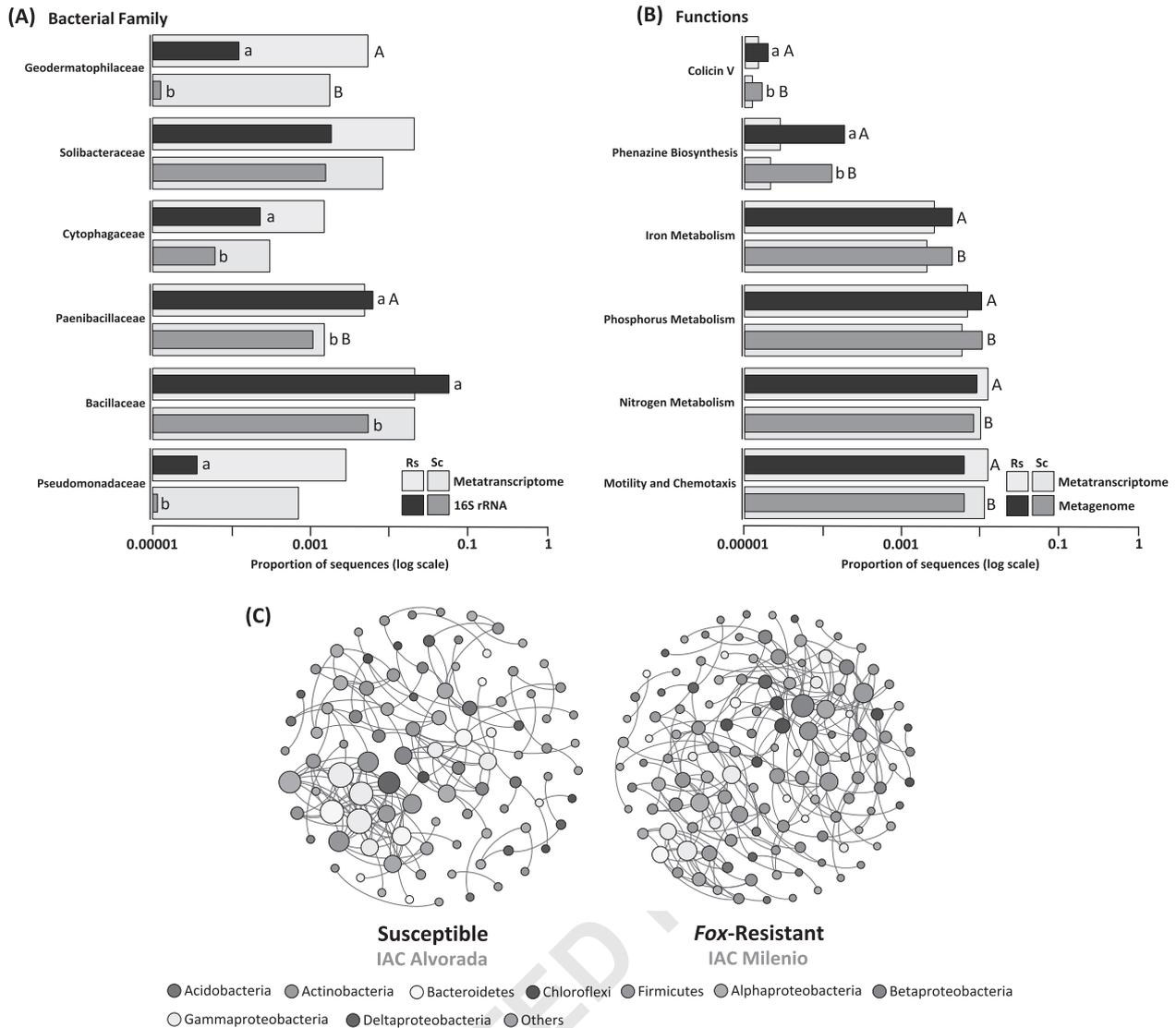


**Fig. 1** Structure and composition of the microbial communities in the rhizosphere of two common bean cultivars with contrasting levels of resistance to *F. oxysporum*. **a** Redundancy analysis (RDA) performed on taxonomic profile (genus level) and environmental characteristics. Arrows indicate correlation between environmental parameters and community structure. Only significant correlations evaluated via the Monte Carlo permutation test ( $P < 0.05$ ) are shown; **b** Principal component analysis (PCA) based on functional profile (SEED database). **c** Heatmap showing the differential abundance of phylum and

family and **d** heatmap for functional categories. The color key relates the heatmap colors to the standard score (z-score), i.e., the deviation from row mean in units of standard deviations above or below the mean. Different lower case letters refer to significant differences between the treatments based on Welch's *t*-test with Benjamini-Hochberg correction ( $P < 0.05$ ). Significant clusters (ANOSIM,  $P < 0.05$ ) are indicated by lines in the graph B (color figure online)

54 Our results showed high microbial activity in the rhizo-  
55 sphere of both cultivars, with parameters such as pH ( $F =$   
56  $3.81$ ,  $P < 0.05$ ), magnesium ( $F = 6.30$ ,  $P < 0.005$ ), and  
57 copper ( $F = 6.23$ ,  $P < 0.005$ ) correlated with the overall  
58 community structure (Fig. 1a and Supplementary Table 1).  
59 Although the resolution obtained in taxonomical classifi-  
60 cation from the metatranscriptomic data did not show dis-  
61 tinct communities between the two cultivars, the functional  
62 profile revealed a clear cultivar-specific clustering of the

63 samples (Fig. 1b). For both cultivars, the rhizosphere pre-  
64 sented high activity of several taxa known as plant growth-  
65 promoting bacteria, such as the families Bradyrhizobiaceae,  
66 Rhizobiaceae, Rhodospirillaceae (Fig. 1c). Comparing the  
67 contrasting bean cultivars, we found higher activity of taxa  
68 affiliated to Paenibacillaceae in the *Fox*-resistant rhizo-  
69 sphere. Several members of this bacterial family are well  
70 known for the production of antibiotics with inhibitory  
71 activity against phytopathogenic fungi [9]. Within this



**Fig. 2** **a** Taxonomic and **b** functional comparison between metatranscriptome data from this study and DNA-based data from Mendes et al. [7]. Different lower case letters indicate significant differences ( $P < 0.05$ ) within DNA-based data, while upper case letters indicate differences within RNA-based data. **c** Network co-occurrence analysis of microbial communities in the rhizosphere of two common bean cultivars with contrasting levels of resistance to *F. oxysporum*. Each node represents a microbial phylotype (*Bacteria* or *Archaea*) at genus level.

A connection stands for SparCC correlation with a magnitude  $>0.7$  (positive correlation—blue edges) or  $<-0.7$  (negative correlation—red edges) and statistically significant ( $P < 0.01$ ). The size of each node is proportional to the number of connections (that is, degree). Each node was labeled at the phylum level, except for Proteobacteria phylum, shown at the class level. Rs = *Fox*-resistant cultivar; Sc = susceptible cultivar (color figure online)

72 family, the genus *Paenibacillus* was found abundantly in  
 73 the rhizosphere of the *Fox*-resistant cultivar (Supplementary  
 74 Fig. 1). This genus is widely distributed in the environment  
 75 and known as an antagonist of soil-borne pathogens,  
 76 including *Fusarium* [9–11]. Comparing these results with  
 77 those from our previous DNA-based analyses [7], we found  
 78 the same patterns/dynamics for all bacterial families and  
 79 functions detected in both studies (Fig. 2a). Interestingly,  
 80 we detected in both datasets (DNA and RNA) a higher  
 81 abundance of bacterial groups belonging to the order  
 82 Bacillales and the family Geodermatophilaceae in the *Fox*-

resistant cultivar when compared with the susceptible cul- 83  
 84 tivar. Species of Bacillales are known for the production of  
 85 antimicrobial compounds such as bacteriocins, non-  
 86 ribosomally synthesized peptides (NRPs), polyketides  
 87 (PKs) and lipopeptides (LPs) [12]. Members of Geo-  
 88 dermatophilaceae play a vital role in several biogeochemi-  
 89 cal processes and are characterized as plant growth-  
 90 promoting rhizobacteria [13]. Our data also revealed that  
 91 functions related to the metabolism of nitrogen, phosphorus,  
 92 iron, nucleotides sugars and transcriptional regulation were  
 93 significantly more expressed in the rhizosphere of the *Fox*-

resistant cultivar (Fig. 1d and Supplementary Tables 2 and 3). A higher abundance of genes related to nutrient metabolism could promote plant growth and health, and healthier plants are more prone to fend off pathogen invasions [14]. Also, the resistant cultivar presented more expression of cell motility and chemotaxis genes, which functions are important for the assembly of the rhizosphere microbiome, as they propel microbial populations to available resources [15]. This ability to move also provides a competitive advantage in the rhizosphere, thereby hampering pathogen invasion.

Further exploration of the data revealed higher expression of genes involved in phenazine and colicin V biosynthesis in the *Fox*-resistant rhizosphere microbiome (Fig. 1d). Phenazine antibiotics have strong activity against *F. oxysporum* in several crops and contribute to Fusarium-wilt suppressiveness of soils [16]. Colicin V (colV) is a secreted peptide antibiotic capable to kill bacterial cells, thereby reducing competition for nutrients [17]. In a recent study, the production of colV by an *Enterobacter* sp. strain was related to the suppression of *Fusarium* wilt in finger millet [18]. Interestingly, genes involved in the biosynthesis of these two ‘anti-*Fusarium*’ compounds were also found more abundantly in the rhizosphere of the *Fox*-resistant bean based on the metagenome data (Fig. 2b). Summarizing, our results suggest that the rhizosphere of the *Fox*-resistant cultivar is enriched with beneficial taxa and functional traits that help the plant to fend off the pathogen. This hypothesis is further supported by the results of the co-occurrence network analysis based on the active community, which showed a more complex network for the *Fox*-resistant cultivar, based on the high number of correlations, high modularity, small diameter, and low average path length (Fig. 2c and Supplementary Table 4). A more complex network has been proposed to better resist pathogen invasions, because of the higher number of species interactions and intensified competition for niche space [15, 19]. To infect root tissue, the fungi have to compete with members of the rhizosphere microbiome for nutrients and microsites [5]. Using the metrics of the network topology we also identified the genus *Paenibacillus* as key taxon in the *Fox*-resistant co-occurrence network with high betweenness centrality and high correlations, which can be interpreted as keystone species within a community (Supplementary Table 5). As discussed above, this bacterial genus is widely known for its activity against *Fusarium*. A pathogen invader may manage to displace the key taxa and collapse the network structure [20]. For example, it was demonstrated that *F. oxysporum* produces fusaric acid, which down regulates the production of the antibiotic compound DAPG in *Pseudomonas*, a key factor for its antagonistic activity in rhizosphere [21]. Although we have identified a key taxon in the network of the *Fox*-resistant

cultivar, the microbiome is more complex and dynamic. Several other microorganisms that can confer suppressiveness have been found among other bacterial phyla, such as Proteobacteria and Firmicutes [22]. The putative beneficial effects of these and other identified bacterial taxa will be subject of future studies.

In light of our results, we suggest that breeding for resistance in common bean have selected a specific active microbial community which presented high expression of beneficial microbial traits that may complement disease protection in addition to the resistance traits of the plant itself. Our study reinforces the importance of understanding the processes of microbiome assembly in the rhizosphere, where the identification of microbial groups and traits related to pathogen suppression could help the future development of plant breeding to select for plant traits that enrich and activate beneficial microbial groups and genes that will protect plants against root pathogens.

**Acknowledgements** This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2014/03217-3, 2015/00251-9) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES 88887.185941/2018-00). Publication XXXX of the Netherlands Institute of Ecology (NIOO-KNAW).

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

- Mendes R, Garbeva P, Raaijmakers JM. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiol Rev.* 2013;37:634–63.
- Bulgarelli D, Garrido-Oter R, Münch PC, Weiman A, Dröge J, Pan Y, et al. Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell Host Microbe.* 2015;17:392–403.
- Mendes LW, Kuramae EE, Navarrete AA, van Veen JA, Tsai SM. Taxonomical and functional microbial community selection in soybean rhizosphere. *ISME J.* 2014;8:1577–87.
- Meena KK, Sorty AM, Bitla UM, Choudhary K, Gupta P, Pareek A, et al. Abiotic stress responses and microbe-mediated mitigation in plants: the omics strategies. *Front Plant Sci.* 2017;8:172.
- Chapelle E, Mendes R, Bakker PAH, Raaijmakers JM. Fungal invasion of the rhizosphere microbiome. *ISME J.* 2016;10:265–8.
- Lebeis SL, Paredes SH, Lundberg DS, Breakfield N, Gehring J, McDonald M, et al. Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science.* 2015;349:860–4.
- Mendes LW, Raaijmakers JM, Hollander M, Mendes R, Tsai SM. Influence of resistance breeding in common bean on rhizosphere microbiome composition and function. *ISME J.* 2018;12:212–24.
- Yao H, Wu F. Soil microbial community structure in cucumber rhizosphere of different resistance cultivars to fusarium wilt. *FEMS Microbiol Ecol.* 2010;72:456–63.

- 201 9. Li P, Ma L, Feng YL, Mo MH, Yang FX, Dai HF, et al. Diversity 228  
202 and chemotaxis of soil bacteria with antifungal activity against 229  
203 *Fusarium wilt* of banana. *J Ind Microbiol Biotechnol.* 230  
204 2012;39:1495–505. 231
- 205 10. Budi SW, van Tuinen D, Arnould C, Dumas-Gaudot E, 232  
206 Gianinazzi-Pearson V, Gianinazzi S. Hydrolytic enzyme activity 233  
207 of *Paenibacillus* sp. strain B2 and effects of the antagonistic 234  
208 bacterium on cell integrity of two soil-borne pathogenic fungi. 235  
209 *Appl Soil Ecol.* 2000;15:191–9. 236
- 210 11. Dijksterhuis J, Sanders M, Gorris LG, Smid EJ. Antibiosis plays a 237  
211 role in the context of direct interaction during antagonism of 238  
212 *Paenibacillus polymyxa* towards *Fusarium oxysporum*. *J Appl* 239  
213 *Microbiol.* 1999;86:13–21. 240
- 214 12. Zhao X, Kuipers OP. Identification and classification of known 241  
215 and putative antimicrobial compounds produced by a wide variety 242  
216 of *Bacillales* species. *BMC Genom.* 2016;17:882. 243
- 217 13. Xiao X, Fan M, Wang E, Chen W, Wei G. Interactions of plant 244  
218 growth-promoting rhizobacteria and soil factors in two legumi- 245  
219 nous plants. *Appl Microbiol Biotechnol.* 2017;101:8485–97. 246
- 220 14. Spann TM, Schumann AW. The role of plant nutrients in disease 247  
221 development with emphasis on citrus and huanglongbing. *Proc* 248  
222 *Flor Stat Hortic Soc.* 2009;122:169–71. 249
- 223 15. Mallon CA, van Elsas JD, Salles JF. Microbial invasions: the 250  
224 process, patterns, and mechanisms. *Trends Microbiol.* 251  
225 2015;23:719–29. 252
- 226 16. Mazurier S, Corberand T, Lemanceau P, Raaijmakers JM. Phe- 253  
227 nazine antibiotics produced by fluorescent pseudomonads  
contribute to natural soil suppressiveness to *Fusarium wilt*. *ISME* 228  
*J.* 2009;3:977–91. 229
- 230 17. Gérard F, Pradel N, Wu L-F. Bactericidal activity of Colicin V Is 230  
231 mediated by an inner membrane protein, SdaC, of *Escherichia* 231  
232 *coli*. *J Bacteriol.* 2005;187:1945–50. 232
- 233 18. Mousa WK, Shearer C, Limay-Rios V, Ettinger CL, Eisen JA, 233  
234 Raizada MN. Root-hair endophyte stacking in finger millet creates 234  
235 a physicochemical barrier to trap the fungal pathogen *Fusarium* 235  
236 *graminearum*. *Nat Microbiol.* 2016;1:16167. 236
- 237 19. Wei Z, Yang T, Friman V-P, Xu Y, Shen Q, Jousset A. Trophic 237  
238 network architecture of root-associated bacterial communities 238  
239 determines pathogen invasion and plant health. *Nat Comm.* 239  
240 2015;6:8413. 240
- 241 20. Albrecht M, Padrón B, Bartomeus I, Traveset A. Consequences of 241  
242 plant invasions on compartmentalization and species' roles in 242  
243 plant-pollinator networks. *Proc Biol Sci.* 2014;281:20140773. 243
- 244 21. Notz R, Maurhofer M, Dubach H, Haas D, Défago G. Fusaric 244  
245 acid-producing strains of *Fusarium oxysporum* alter 2,4-diacety- 245  
246 lphloroglucinol biosynthetic gene expression in *Pseudomonas* 246  
247 *fluorescens* CHA0 in vitro and in the rhizosphere of wheat. *Appl* 247  
248 *Environ Microbiol.* 2002;68:2229–35. 248
- 249 22. Raaijmakers JM, Paulitz TC, Steinberg C, Alabouvette C, 249  
250 Moënne-Loccoz Y. The rhizosphere: a playground and battlefield 250  
251 for soilborne pathogens and beneficial microorganisms. *Plant Soil.* 251  
252 2009;321:341–61. 252

UNCORRECTED PROOF

Journal : 41396

Article : 234

**SPRINGER NATURE**

## Author Query Form

**Please ensure you fill out your response to the queries raised below and return this form along with your corrections**

Dear Author

During the process of typesetting your article, the following queries have arisen. Please check your typeset proof carefully against the queries listed below and mark the necessary changes either directly on the proof/online grid or in the 'Author's response' area provided below

Queries	Details Required	Author's Response
AQ1	Author surnames have been highlighted - please check these carefully and indicate if the first name or surname have been marked up incorrectly. Please note that this will affect indexing of your article, such as in PubMed.	
AQ2	Please provide publication no. for "Publication XXXX of the Netherlands Institute of Ecology (NIOO-KNAW)".	

UNCORRECTED PROOF