Biol Fertil Soils (2014) 50:217–224
DOI 10.1007/s00374-013-0835-1

ORIGINAL PAPER

De-coupling of root–microbiome associations followed by antagonist inoculation improves rhizosphere soil suppressiveness

Meihua Qiu · Shuqing Li · Xuan Zhou · Xiaoshuang Cui · Jorge M. Vivanco · Nan Zhang · Qirong Shen · Ruifu Zhang

Received: 1 April 2013 / Revised: 24 June 2013 / Accepted: 1 July 2013 / Published online: 23 August 2013

Abstract It was hypothesized that disruption of the root–microbiome association creates empty rhizosphere niches that could be filled by both soilborne pathogens and beneficial microbes. The effect of de-coupling root–microbiome associations related to improve soil suppressiveness was investigated in cucumber using the pathogen *Fusarium oxysporum* f. sp. *Cucumerinum* (FOC) and its antagonist *Bacillus amyloliquefaciens* SQR9 (SQR9) system. The root–soil microbiome association of cucumber was disrupted by applying the fungicide carbendazim to the soil, and then FOC or/and its antagonist SQR9 were inoculated in the rhizosphere. In the fungicide treatment, the FOC wilt disease incidence was significantly increased by 13.3 % on average compared to the FOC treatment without fungicide. However, when the fungicide treatment was applied to the soil with SQR9 and FOC, the SQR9 effectively reduced the disease incidence, and improved cucumber plant growth compared to a no fungicide control. These results indicate that de-coupling of root–microbiome associations followed by antagonist inoculation can improve rhizosphere soil suppressiveness, which may help to develop strategies for efficient application of rhizosphere beneficial microbes in agriculture.

Keywords Root–microbiome association · Rhizosphere niche · Soil suppressiveness · *Fusarium* wilt

Meihua Qiu and Shuqing Li contributed equally to this paper

Electronic supplementary material The online version of this article (doi:10.1007/s00374-013-0835-1) contains supplementary material, which is available to authorized users.

M. Qiu · S. Li · X. Zhou · X. Cui · N. Zhang · Q. Shen · R. Zhang
Key Laboratory of Plant Nutrition and Fertilization in Low-Middle Reaches of the Yangtze River, Ministry of Agriculture, 210095 Nanjing, People's Republic of China

M. Qiu · S. Li · X. Zhou · X. Cui · N. Zhang · Q. Shen · R. Zhang (✉)
Jiangsu Key Lab and Engineering Center for Solid Organic Waste Utilization, College of Resources and Environmental Science, Nanjing Agricultural University, 210095 Nanjing, People's Republic of China
e-mail: rfzhang@njau.edu.cn

J. M. Vivanco
Center for Rhizosphere Biology, Colorado State University, Fort Collins, CO 80523-1173, USA

J. M. Vivanco (✉)
Department of Horticulture and Landscape Architecture, Colorado State University, Fort Collins, CO 80523, USA
e-mail: j.vivanco@colostate.edu

Introduction

Plants live in close association with microbes that inhabit the soil. The rhizosphere, which is the narrow zone of soil that is directly influenced by plant roots and root exudates, can contain up to 10^{11} microbial cells per gram of root (Egamberdieva et al. 2008) and more than 30,000 prokaryotic species (Mendes et al. 2011). It is regarded as an important and active zone for microbial colonization and activity (Hiltner 1904; Hartmann et al. 2008). The collective genome of the rhizosphere microbes is much larger than that of the plant and is also referred to as the plant's second genome (Berendsen et al. 2012).

Both soil type and plant genotype have important effects on the composition of the rhizosphere microbiome (Garbeva et al. 2008; Berg and Smalla 2009; Yao and Wu 2010; Bever et al. 2012), which consists of the entire community of rhizosphere-associated microbes and their specialized functions. Microbial communities in the rhizosphere of different plant species growing on the same soil are usually distinct (Berg et al. 2006; Garbeva et al. 2008) but some plant species develop their own core microbiome even when grown in different soils (Bulgarelli et al. 2012; Lundberg et al. 2012);

thus suggesting that plants actively shape the composition of the microbiome in their rhizosphere. The interactions between roots and the rhizosphere microbiome are often specialized and based on co-evolutionary pressures (Dobbelaere et al. 2003; Duffy et al. 2004; Morrissey et al. 2004; Morgan et al. 2005; Broeckling et al. 2008). Moreover, several reports also indicated that the microbial communities in the rhizosphere soil are sometimes determined by the bulk soil community (de Ridder-Duine et al. 2005; Nannipieri et al. 2008a, b).

An increasing body of evidence highlights the importance of the root microbiome in determining plant health and productivity (Berendsen et al. 2012). The rhizosphere microbiome can help plants in disease suppression (Mendes et al. 2011), nutrient uptake (Lugtenberg et al. 2002; Morrissey et al. 2004), and abiotic (Selvakumar et al. 2012) and biotic stress tolerance by increasing plant immunity (Zamioudis and Pieterse 2012) thus leading to increases in plant productivity (Berg 2009). In turn, the plant provides the soil microbes with root exudates that are used as substrates and signaling molecules (Badri and Vivanco 2009; Badri et al. 2009).

The microflora of most soils is starved and as a result there is competition in the rhizosphere between microbes for plant-derived nutrients (Raaijmakers et al. 2009). Most soilborne pathogens grow saprophytically in the rhizosphere to reach and infect their host, and their success in the colonization of the roots is influenced by competition with the native rhizosphere microbiome (Bakker et al. 2012; Chaparro et al. 2012). Thus, fostering a healthy soil microbiome limits pathogen success. Suppressive soils are characterized by their ability to limit the growth and activity of soilborne pathogens and consequently plant disease. Such suppressiveness is typically dependent upon the activity of resident beneficial or antagonistic microbes (Weller et al. 2002) with increasing microbial biomass and/or diversity (Larkin and Honeycutt 2006; Postma et al. 2008). Evenness of the rhizosphere microbiome is an important factor for soil suppression (Crowder et al. 2010; Yao and Wu 2010) ensuring that no individual microbial taxum is dominant. A diverse and even microbiome maximizes niche overlap between pathogens and other community members in the rhizosphere limiting empty niche space for potential invaders and new comers (Hillebrand et al. 2008). Besides this background information on suppressive soils, there is limited information available in the literature about methodologies to promote suppression in agricultural soil systems.

In modern agriculture, management practices such as the application of pesticides, fungicides, and fertilizers often disrupt the root–microbiome associations (Postma-Blaauw et al. 2010) resulting in constant adjustments in rhizosphere niche availability. These constant disruptions in agricultural systems may not allow sufficient time for a diverse microbiome to develop that includes pathogen antagonists. It was hypothesized that these disruptive soil management strategies may create an opportunity for pathogen establishment for colonization by taking advantage of the de-coupling of plant–microbiome associations (Bakker et al. 2012). Similarly, this disruption could be exploited as an opportunity to successfully introduce beneficial organisms. Thus, we hypothesized that intentional disruption of root–microbiome associations followed by amendments with beneficial microbes can improve the soil suppressiveness. To test this hypothesis and in an effort to develop suppressiveness in soils, we treated the cucumber rhizosphere with a fungicide to partially disrupt the associations of cucumber root-fungi, and followed this treatment by the application of *Fusarium oxysporum* f. sp. *Cucumerinum* (FOC), the soilborne pathogen of cucumber wilt disease, and *Bacillus amyloliquefaciens* SQR9 (SQR9), an efficient antagonist against FOC (Cao et al. 2011; Weng et al. 2013; Xu et al. 2013).

Materials and methods

Strains and growth conditions

The pathogen FOC was incubated on potato dextrese agar solid media at 28 °C for 10 days. Plates were washed with sterile distilled water and spores were scraped from the medium surface with a sterile cover slip. The spore suspension was then filtered through three layers of sterile gauze to eliminate mycelial fragments. The conidia concentration was calculated by hemacytometer.

The antagonistic SQR9 bacteria isolated from cucumber rhizosphere (Cao et al. 2011; Weng et al. 2013; Xu et al. 2013) was incubated in Luria Bertani liquid medium at 37 °C and 170 rpm for 48 h. The SQR9 culture was centrifuged at 6,000×*g* at 4 °C for 10 min and the cell pellet obtained was washed three times in sterile distilled water and resuspended in it. The concentration of SQR9 suspension was determined by serial dilution plate counting method.

Pot experimental design

The trial was conducted from July to September 2012, in the greenhouse of Nanjing Agricultural University. The soils used for the pot experiments were collected from a field site with a history of cucumber cultivation. The field site was located in Nanjing, Jiangsu Province, China and the soil had the following properties: pH 5.41, organic matter 23.2 g kg^{-1}, available N 159 mg kg^{-1}, available P 138 mg kg^{-1}, available K 272- mg kg^{-1}, total N 1.92 g kg^{-1}, total P 1.97 g kg^{-1}, and total K 16.4 g kg^{-1}.

Seeds of cucumber, Jinchun No.4, were surface-disinfested in 2 % sodium hypochlorite for 3 min, rinsed three times in sterile distilled water, and then germinated in 9-cm petri dishes covered with sterile wet filter paper at 30 °C. After germinating, the seeds were planted in seedling trays. When the cucumber seedlings had two true leaves, they were divided into two

groups. Group A: without application of the fungicide carbendazim (Car) to the seedling rhizosphere (control group; CK). Group B: addition of carbendazim (final concentration 10 mg kg^{-1} soil) to the cucumber seedling rhizosphere in the root irrigation way. After cultivation for 7 days, the seedlings were transplanted to the pot. The soils (1 kg in each pot) in the pots, were inoculated with SQR9 or/and FOC. Four treatments for group A (CK-control, FOC-pathogen, SQR9-antagonist, and FOC + SQR9) and group B (Car-CK, Car-FOC, Car-SQR9, and Car-FOC + SQR9) were designed. FOC was inoculated at a concentration of 10^5 spore g^{-1} soil. SQR9 was inoculated at a concentration of 10^8 cfu g^{-1} soil.

Each treatment was replicated 30 times, which included three blocks in a completely randomized design (ten plants for each block). The seedlings were incubated in a growth chamber at 30 °C under a 16-h light regimen and irrigated with 1/2 Hoagland medium.

The bioassay of disease incidence was performed 2-months after transplanting and expressed as the percentage of diseased plants over the total number of growing plants in each block.

Soil sampling and DNA extraction

The soil for subsequent analysis was collected from the cucumber rhizosphere of ten plants in each block when the *Fusarium* wilt appeared. Soil DNA was extracted from 1 g of the mixed soil samples, obtained by combining ten replicates, using the UltraClean™ Soil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) and according to the manufacturer's instruction. Ten DNA extractions of each soil sample were pooled.

Quantification of FOC and SQR9 by real-time PCR

Real-time PCR amplification and detection was performed on Applied Biosystems 7500 Real-Time PCR system using Premix Ex Taq™ kit (Takara, Dalian, China). The primers for detection of FOC were FocF3 (F) 5′-AAACGAGCCCGCTATTTGAG-3′ and FocR7 (R) 5′-TATTTCCTCCACATTGCCATG-3′ (Lievens et al. 2007). A 10-fold serial dilution of the plasmid pMD-FOC (the product of FocF3 and FocR7 ligated with pMD-19 T, 5.52×10^2 to 5.52×10^6 copies) and primer pair FocF3 (F)/FocR7 (R) was used to construct the standard curve. The equation of the standard curve: $y=-3.2679x+38.654$, $R^2=0.9992$, $E=102$ %, x represents the value of threshold cycles (Ct) and y shows the number of FOC (Log_{10} copies). The primers for specific detection of SQR9 were SQR9F (F) 5′-CATGAGATGGCGGGCTTT −3′ and SQR9R (R) 5′- CGCATCCTCCCTGTCTTTG-3′, which were designed according to the comparative analysis of its genome sequence (unpublished) with genomes of other *Bacillus* strains, and the specificity of the primers was tested (supplemental materials). A 10-fold serial dilution of the plasmid pMD-unknown (the product of SQR9F and SQR9R ligated with pMD19-T, 9.99×10^2 to 9.99×10^7 copies) and primer pair SQR9F (*F*)/SQR9R (*R*) was used to construct the standard curve. The equation of the standard curve: $y=-3.1396x+39.299$, $R^2=0.9949$, $E=108$ %, x represents the value of Ct and y shows the number of SQR9 (Log_{10} copies, Fig. S1).

Real-time PCR amplification was prepared in triplicates in MicroAmp Optical 96-well reaction plates and sealed with optical adhesive covers (Applied Biosystems). A typical real-time PCR reaction mixture was prepared, which contained 10.0-μl *Premix Ex Taq*™ (2×), 0.5 μM of each primer, 0.4-μl ROX Reference Dye II (×50), 20 ng DNA template, and a final volume of 20 μl with sterile water. The PCR program consisted of an initial denaturation at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 5 s, and annealing and extension at 60 °C for 34 s. Ct values was automatically performed by the system. The melt curve analysis was conducted at the end of the PCR run to evaluate amplification specificity.

Total DNA from soil samples of different treatments was subjected to the real-time PCR assay to quantify the total FOC and SQR9 copy numbers.

Statistical analysis

Differences among the eight treatments were calculated and statistically analyzed with one-way analysis of variance (ANOVA). Duncan's multiple-range test was used when the one-way ANOVA indicated significant differences ($P<0.05$). All statistical analysis was carried out with SPSS BASE ver.11.5 statistical software (SPSS, Chicago, IL, USA).

Results

Effects of root–microbiome de-coupling on soil suppressiveness

The effect of de-coupling root–microbiome associations related to improve soil suppressiveness was investigated in pot experiments as described in the M&M. The number of fungi in the rhizosphere soil decreased almost 10-fold after the soil was treated with carbendazim. Moreover, carbendazim inhibited the growth of the pathogen FOC (data not shown). However, the population of bacteria was kept stable after treatment of carbendazim.

Results of cucumber disease incidence between groups with and without fungicide are shown in Fig. 1. There were significant differences among the four treatments in each group. The disease incidence of FOC (pathogen) treatment was on average of 63.3 %. Treatment Car-FOC (fungicide + pathogen) showed

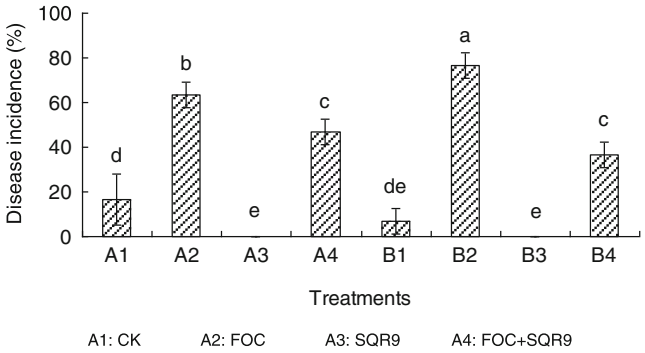

Fig. 1 The disease incidence rate of cucumber *Fusarium* wilt. *Letters* above the bars indicate a significant difference according to Duncan's multiple-range test at a $P < 0.05$ level

an average disease incidence of 76.6 %. The FOC + SQR9 (pathogen + antagonist) showed 46.6 % disease incidence in comparison with 36.6 % in the treatment Car-FOC + SQR9 (fungicide + pathogen + antagonist).

Effects of root–microbiome de-coupling on plant growth

Besides soil pathogen suppression, SQR9 can also promote plant growth through the production of phytohormones, volatile chemicals, and phosphorus solubilization (unpublished data). Plant growth parameters, shoot height, and plant biomass suggested significant differences among the treatments. Car-SQR9 treatment effectively promoted the cucumber growth compared to the treatments SQR9 and the controls CK and Car-CK (Table 1). However, the treatment Car-FOC showed significant lower levels of growth than FOC and the controls. Overall, the fungicide treatments to the soil improved plant growth when SQR9 was added but had the contrary effect when FOC was inoculated compared to their respective non disrupted (not treated with fungicide) treatments (Fig. S2).

Quantification of FOC and SQR9 in cucumber rhizosphere by real-time PCR

Real-time quantitative assays were used to determine the FOC and SQR9 copy numbers of the target DNA present in the rhizosphere soil of cucumber plants in the different treatments. There was a significant difference of FOC distribution between the treatments with or without application of carbendazim. The DNA numbers (Log_{10} Copies) of FOC in the treatments with and without fungicides were 5.62 and 6.11, respectively (Table 2). Treatments FOC + SQR9 and Car-FOC + SQR9 showed a significant FOC number decrease compared with FOC and Car-FOC treatments, which indicated that SQR9 could inhibit the growth of FOC. The quantity of SQR9 in the Car-SQR9 soil was 5.75, which was significantly higher than that in the SQR9 treatment without carbendazim amendment. Compared with these two treatments, less SQR9 was found in the treatments FOC + SQR9 and Car-FOC + SQR9, which suggested that FOC could, to some extent, influence SQR9 growth in the cucumber rhizosphere. The populations of SQR9 in the four treatments of CK, FOC, Car-CK, and Car-FOC could not be detected thus suggesting that the antagonist was not present in the cucumber soil.

Discussion

Microorganisms are associated with above- and below-ground parts of living plants, and they are considered harmful or beneficial, depending on whether they depress or favor plant growth and health (Marx 2004). It was hypothesized that disturbance of root–microbiome associations shifts the previous structure of microbial community in the rhizosphere; thus allowing the colonization by new comers. In our experiment, we applied the fungicide carbendazim (Group B) to the cucumber rhizosphere disturbing the original root–microbiome association by killing large numbers of fungi (data not shown) and releasing empty rhizosphere niches that were colonized by either the pathogen or the antagonistic microbes.

As disease suppressiveness can be viewed as a manifestation of ecosystem stability and health (van Bruggena and Semenovb 2000), we investigated the disease incidence by FOC of cucumber plants among different treatments. Since the soils used for this study were collected from a field site with a history of cucumber monoculture, slight wilt disease

Table 1 Effect of different treatments on plant growth of cucumber

Treatments	Shoot height (cm plant^{-1})	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})
CK	96.2±5.86c	5.97±0.23d	0.38±0.09d
FOC	84.4±3.06d	4.71±0.65e	0.26±0.03e
SQR9	123±4.97b	9.15±0.59b	0.99±0.08a
FOC + SQR9	97.5±3.82c	6.50±0.19 cd	0.48±0.06 cd
Car-CK	99.0±5.72c	6.53±0.20 cd	0.79±0.03b
Car-FOC	71.3±3.50e	4.41±0.18e	0.20±0.04e
Car-SQR9	148±4.49a	11.1±0.46a	1.05±0.09a
Car-FOC + SQR9	110±7.14c	7.16±0.30c	0.58±0.05c

Data were expressed as mean ± standard error. Mean values in the same column followed by the same letter are not significantly different at Duncan's significance level of 0.05

Table 2 Quantification of FOC and SQR9 in cucumber rhizosphere soil using real-time PCR

Treatments	*Fusarium oxysporum* f. sp. *Cucumerinum* (FOC)		*Bacillus amyloliquefaciens* SQR9	
	Ct value	Log10 Copies	Ct value	Log10 Copies
CK	25.3±0.21d	4.10±0.06e	31.7±0.09a	–
FOC	20.3±0.15 g	5.62±0.04b	31.5±0.17ab	–
SQR9	31.2±0.14b	–	22.4±0.32d	5.39±0.10b
FOC + SQR9	22.0±0.17f	5.09±0.05c	25.3±0.14c	4.46±0.04c
Car-CK	26.5±0.13c	3.72±0.04f	31.4±0.25ab	–
Car-FOC	18.7±0.12 h	6.11±0.04a	31.3±0.19b	–
Car-SQR9	31.9±0.11a	–	21.3±0.12e	5.75±0.04a
Car-FOC + SQR9	23.1±0.26e	4.76±0.08d	25.1±0.08c	4.52±0.03c

Data were expressed as mean ± standard error. Mean values in the same column followed by the same letter are not significantly different at Duncan's significance level of 0.05. Ct value over 30 represented an inefficient PCR amplification (Supplemental Information)

occurred in the control treatments (CK and Car-CK). The highest disease incidence (76.6 %) occurred in the Car-FOC treatment (Fig. 1), which was probably attributed to the empty niche after fungicide treatment occupied by the pathogen FOC in the cucumber rhizosphere. Accordingly, Stachowicz and Tilman (2005) suggested that species-poor regions had greater resource availability and were more easily invaded.

When SQR9 was inoculated in the Car-FOC treatment namely Car-FOC + SQR9, the disease incidence significantly decreased (Fig. 1). This result was in accordance with other reports (Cao et al. 2011; Qiu et al. 2012) as SQR9 could effectively suppress the wilt disease. Application of SQR9 in the Car-FOC + SQR9 treatment possibly competed with the pathogen for the vacant niches created upon fungicide treatment. Real-time PCR can detect and quantify not only non-culturable but also slow-growing microorganisms (Gao et al. 2004; Hermansson et al. 2004; Jørgensen and Leser 2007). In our study, the highest copy number of FOC detected was present in the treatment of Car-FOC (Table 2), which was in accordance with the disease incidence. Like application of carbendazim increased niche vacancy and the introduced FOC pathogen effectively colonized the cucumber rhizosphere, a similar situation occurred with the antagonist SQR9 in the Car-SQR9 treatment.

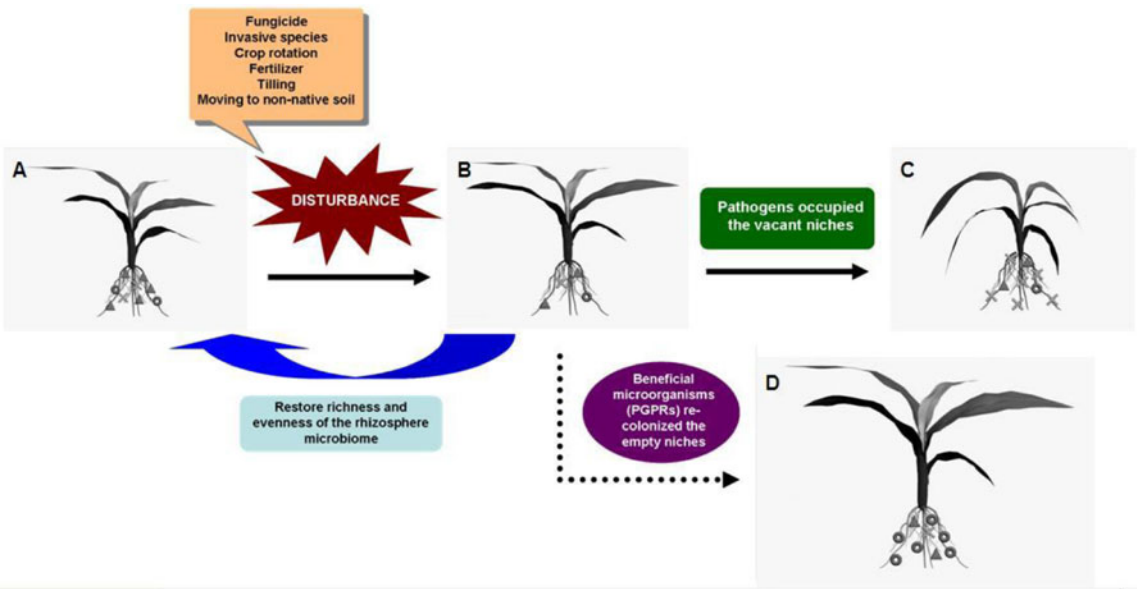

Fig. 2 Visual model representing different types of root–microbiome association. Symbols with different shapes below the soil line represent different microbial taxa: *X* pathogenic taxa, *Circle* beneficial taxa, and *Triangles* other taxa. **a** A plant growing in a location where there has been a long history of co-adaptation with the rhizosphere microbiome. Although pathogens are present, their activity is greatly constrained. **b** Due to disturbance by agricultural management, the root–microbiome association was de-coupled and empty rhizosphere niches were released. The diversity and evenness of the rhizosphere microbiome may be restored through management, such as organic amendment. **c** The vacant niches may also be occupied by introduced or present soilborne pathogens, leading to disease. **d** However, the disrupted root–microbiome association and the empty rhizosphere niches can be utilized by carefully planned management techniques, including application of beneficial microorganisms (PGPRs), and improves the plant health and growth

Ecologists have recognized that continued persistence of multiple species requires some minimum differentiation in niches and that overlap in niches can prevent species from establishing (Case 1983; Diamond 1975). Long-term associations under relatively natural conditions have led to co-adaptation between root and its associated microbiome (Fig. 2a). Niche occupancy in the rhizosphere under these circumstances is likely to be maximized because sufficient time has passed for adaptation and evolution among members of the microbiome, such that available resources are fully exploited. Thus, it is assumed that overlapping rhizosphere niches for soilborne pathogens and their antagonist microbes creates a strong competition for the same resource base, and constrains pathogen activity (Bakker et al. 2012).

To fully exploit the advantage of the co-adapted association, agricultural management should minimize the de-coupling of naturally occurring co-adapted root–microbiome system and minimize the creation of empty rhizosphere niches. In modern agricultural systems, however, it is difficult to maintain undisturbed root–microbiome associations for a long time since intensive disturbances on perturbations including crop rotation, tillage, the use of pesticides, fungicides, and fertilizers, contributes to negative impacts on the rhizosphere microbiome (Postma-Blaauw et al. 2010; Sugiyama et al. 2010). Regular changes in soil habitat result in constant adjustments in rhizosphere niche space, which does not allow time for a diverse, uniform microbial community to develop that includes a number of pathogen antagonists (Fig. 2b).

De-coupling root–microbiome association prevents the full utilization of resources in the rhizosphere and facilitates the establishment of soilborne plant pathogens. Pathogens that are present or introduced may face fewer constraints in the rhizosphere, thus an unintended effect of de-coupling root–microbiome co-adaptation, through regular disturbances, may be to reduce the soil suppressiveness and thus give soilborne pathogens an advantage in the rhizosphere (Fig. 2c). Empty rhizosphere niches could also be exploited as an opportunity to introduce beneficial organisms (plant growth-promoting rhizobacteria (PGPRs)) for re-colonization of empty niches, which would also face fewer restraints in the disturbed rhizosphere (Fig. 2d).

In practical, continuous cropping of the cucumber often lead to reduction of both the yield and quality of the crop (Zhou and Wu 2012a). This phenomenon could be attributed to the secretion of some autotoxins from the cucumber roots, such as *p-coumaric* acid and *p*-hydroxybenzoic acid, which induced alternation of the soil microbial communities, especially the pathogen abundance (Zhou and Wu 2012b; Zhou et al. 2012). Currently, the improvement of soil suppressiveness has been achieved by using wild rocket (*Diplotaxis tenuifolia*) and *Trichoderma* (Chen et al. 2012; Klein et al. 2013). However, Gao et al. (2012) also indicated that the introduction of a biocontrol agent *Pseudomonas fluorescens* 2P24 could only bring a transient effect on soil fungal community, implicating that normal microbial regulation strategy may reveal limited influence on soil community. Here, we provided a novel possible management technique for agricultural application, namely intentionally disturbing the rhizosphere by crop rotations (Peters et al. 2003), fungicide applications, or mechanical disturbances such as tilling (Ibekwe et al. 2002), and then apply PGPRs to minimize niche vacancy and effectively "seal off" the empty rhizosphere niches since PGPRs are strong root colonizers (Cao et al. 2011; Ling et al. 2010, 2013; Qiu et al. 2012; Zhang et al. 2008; Zhao et al. 2011). Incidentally, it has been reported that PGPRs colonize particularly and effectively in soils with low microbial biomass (Fliessbach et al. 2009).

Acknowledgments This research was financially supported by the Chinese Ministry of Science and Technology (2013AA100802) and the National Natural Science Foundation of China (41271271). RZ and QS were also supported by the Priority Academic Program Development of Jiangsu Higher Education Institutions. JMV was supported by a grant from the National Science Foundation (MCB-0950857). RZ, QR, and JMV are part of the 111 Project (B12009).

References

Badri DV, Vivanco JM (2009) Regulation and function of root exudates. Plant Cell Environ 32:666–681

Badri DV, Weir TL, van der Lelie D, Vivanco JM (2009) Rhizosphere chemical dialogues: plant-microbe interactions. Curr Opin Biotech 20(6):642–650

Bakker MG, Manter DK, Sheflin AM, Weir TL, Vivanco JM (2012) Harnessing the rhizosphere microbiome through plant breeding and agricultural management. Plant Soil 360:1–13

Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–486

Berg G (2009) Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotech 84:11–18

Berg G, Opelt K, Zachow C, Lottmann J, Gotz M, Costa R, Smalla K (2006) The rhizosphere effect on bacteria antagonistic towards the pathogenic fungus *Verticillium* differs depending on plant species and site. FEMS Microbiol Ecol 56:250–261

Berg G, Smalla K (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol Ecol 68:1–13

Bever JD, Platt TG, Morton ER (2012) Microbial population and community dynamics on plant roots and their feedbacks on plant communities. Annu Rev Microbiol 66:265–283

Broeckling CD, Broz AK, Bergelson J, Manter DK, Vivanco JM (2008) Root exudates regulate soil fungal community composition and diversity. Appl Environ Microbiol 74:738–744

Bulgarelli D, Rott M, Schlaeppi K, van Themaat EVL, Ahmadinejad N, Assenza F, Rauf P, Huettel B, Reinhardt R, Schmelzer E, Peplies J, Gloeckner FO, Amann R, Eickhorst T, Schulze-Lefert P (2012) Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. Nature 488:91–95

Cao Y, Zhang Z, Ling N, Yuan Y, Zheng X, Shen B, Shen Q (2011) *Bacillus subtilis* SQR9 can control *Fusarium* wilt in cucumber by colonizing plant roots. Biol Fertil Soils 47:495–506

Case TJ (1983) Niche overlap and the assembly of island lizard communities. Oikos 41:427–433

Chaparro JM, Sheflin AM, Manter DK, Vivanco JM (2012) Manipulating the soil microbiome to increase soil health and plant fertility. Biol Fertil Soils 48:489–499

Chen L, Huang X, Zhang F, Zhao D, Yang X, Shen Q (2012) Application of *Trichoderma harzianum* SQR-T037 bio-organic fertilizer significantly controls *Fusarium* wilt and affects the microbial communities of continuously cropped soil of cucumber. J Sci Food Agric 92:2465–2470

Crowder DW, Northfield TD, Strand MR, Snyder WE (2010) Organic agriculture promotes evenness and natural pest control. Nature 466:109–112

Diamond JM (1975) Assembly of species communities. In: Cody ML, Diamond JM (eds) Ecology and evolution of communities. Harvard University Press, Cambridge, MA, pp 342–444

De Ridder-Duine AS, Kowalchuk GA, Klein Gunnewied PJA, Smant W, van Veen JA, de Boer W (2005) Rhizosphere bacterial community composition in natural stands of *Carex arenaria* (sand sedge) is determined by bulk soil community composition. Soil Biol Biochem 37:349–357

Dobbelaere S, Vanderleyden J, Okon Y (2003) Plant growth-promoting effects of diazotrophs in the rhizosphere. Crit Rev Plant Sci 22:107–149

Duffy B, Keel C, Defago G (2004) Potential role of pathogen signaling in multitrophic plant-microbe interactions involved in disease protection. Appl Environ Microbiol 70:1836–1842

Egamberdieva D, Kamilova F, Validov S, Gafurova L, Kucharova Z, Lugtenberg B (2008) High incidence of plant growth-stimulating bacteria associated with the rhizosphere of wheat grown on salinated soil in Uzbekistan. Environ Microbiol 10:1–9

Fliessbach A, Winkler ME, Lutz MP, Oberholzer H, Mäder P (2009) Soil amendment with *Pseudomonas fluorescens* CHA0: lasting effects on soil biological properties in soils low in microbial biomass and activity. Microb Ecol 57:611–623

Gao G, Yin D, Chen S, Xia F, Yang J, Li Q, Wang W (2012) Effect of biocontrol agent *Pseudomonas fluorescens* 2P24 on soil fungal community in cucumber rhizosphere using T-RFLP and DGGE. PLoS One 7:e31806. doi:10.1371/journal.pone.0031806

Gao X, Jackson TA, Lambert KN, Li S, Hartman GL, Niblack TL (2004) Detection and quantification of *Fusarium solani* f. sp. *glycines* in soybean roots with real-time quantitative polymerase chain reaction. Plant Dis 88:1372–1380

Garbeva P, van Elsas JD, van Veen JA (2008) Rhizosphere microbial community and its response to plant species and soil history. Plant Soil 302:19–32

Hartmann A, Rothballer M, Schmid M (2008) Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. Plant Soil 312:7–14

Hermansson A, Backman JSK, Svensson BH, Lindgren PE (2004) Quantification of ammonia-oxidising bacteria in limed and non-limed acidic coniferous forest soil using real-time PCR. Soil Biol Biochem 36:1935–1941

Hillebrand H, Bennett DM, Cadotte MW (2008) Consequences of dominance: a review of evenness effects on local and regional ecosystem processes. Ecology 89:1510–1520

Hiltner L (1904) Über neuere Erfahrungen und Probleme auf dem Gebiete der Bodenbakteriologie unter besonderer Berücksichtigung der Gründüngung und Brache. Arb DLG 98:59–78

Ibekwe AM, Kennedy AC, Frohne PS, Papiernik SK, Yang CH, Crowley DE (2002) Microbial diversity along a transect of agronomic zones. FEMS Microbiol Ecol 39:183–191

Jørgensen C, Leser TD (2007) Estimating amplification efficiency improves multiplex real-time PCR quantification of *Bacillus licheniformis* and *Bacillus subtilis* spores in animal feed. J Microbiol Meth 68:588–595

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

Larkin RP, Honeycutt CW (2006) Effects of different 3-year cropping systems on soil microbial communities and rhizoctonia diseases of potato. Phytopathology 96:68–79

Li S, Zhang N, Zhang Z, Luo J, Shen B, Zhang R, Shen Q (2013) Antagonist *Bacillus subtilis* HJ5 controls *Verticillium* wilt of cotton by root colonization and biofilm formation. Biol Fertil Soils 49:295–303

Lievens B, Claes L, Vakalounakis DJ, Vanachter ACRC, Thomma BPHJ (2007) A robust identification and detection assay to discriminate the cucumber pathogens *Fusarium oxysporum* f. sp. *cucumerinum* and f. sp. *radicis-cucumerinum*. Environ Microbiol 9:2145–2161

Ling N, Xue C, Huang Q, Yang X, Xu Y, Shen Q (2010) Development of a mode of application of bioorganic fertilizer for improving the biocontrol efficacy to *Fusarium* wilt. BioControl 55:673–683

Lugtenberg B, Chin-A-Woeng T, Bloemberg G (2002) Microbe-plant interactions: principles and mechanisms. Antonie Van Leeuwenhoek 81:373–383

Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, Tremblay J, Engelbrektson A, Kunin V, del Rio TG, Edgar RC, Eickhorst T, Ley RE, Hugenholtz P, Tringe SG, Dangl JL (2012) Defining the core *Arabidopsis thaliana* root microbiome. Nature 488:86–90

Marx J (2004) The roots of plant-microbe collaborations. Science 304:234–236

Mendes R, Kruijt M, de Bruijn I, Dekkers E, van der Voort M, Schneider JH, Piceno YM, DeSantis TZ, Andersen GL, Bakker PA, Raaijmakers JM (2011) Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science 332:1097–1100

Morgan JAW, Bending GD, White PJ (2005) Biological costs and benefits to plant-microbe interactions in the rhizosphere. J Exp Bot 56:1729–1739

Morrissey JP, Dow JM, Mark GL, O'Gara F (2004) Are microbes at the root of a solution to world food production? EMBO Rep 5:922–926

Nannipieri P, Ascher J, Ceccherini MT, Guerri G, Renella G, Pietramellara G (2008a) Recent advances in functional genomics and proteomics of plant associated microbes. In: Nautiyal CS, Dion P (eds) Molecular mechanisms of plant and microbe coexistence. Springer, Heidelberg, Germany, pp 215–241

Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G, Valori F (2008b) Effects of root exudates on microbial diversity and activity in rhizosphere soils. In: Nautiyal CS, Dion P (eds) Molecular mechanisms of plant and microbe coexistence. Springer, Heidelberg, Germany, pp 339–365

Peters RD, Sturz AV, Carter MR, Sanderson JB (2003) Developing disease-suppressive soils through crop rotation and tillage management practices. Soil Till Res 72:181–192

Postma-Blaauw MB, de Goede RGM, Bloem J, Faber JH, Brussaard L (2010) Soil biota community structure and abundance under agricultural intensification and extensification. Ecology 91:460–473

Postma J, Schilder MT, Bloem J, van Leeumen-Haagsma WK (2008) Soil suppressiveness and functional diversity of the soil microflora in organic farming systems. Soil Biol Biochem 40:2394–2406

Qiu M, Zhang R, Xue C, Zhang S, Li S, Zhang N, Shen Q (2012) Application of bio-organic fertilizer can control *Fusarium* wilt of cucumber plants by regulating microbial community of rhizosphere soil. Biol Fertil Soils 48:807–816

Raaijmakers JM, Paulitz TC, Steinberg C, Alabouvette C, Moenne-Loccoz Y (2009) The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil 321:341–361

Selvakumar G, Panneerselvam P, Ganeshamurthy AN (2012) Bacterial mediated alleviation of abiotic stress in crops. In: Maheshwari DK (ed) Bacteria in agrobiology: stress management. Springer, Berlin, pp 205–224

Stachowicz JJ, Tilman D (2005) Species invasions and the relationships between species diversity, community saturation, and ecosystem

functions. In: Sax DF, Stachowicz JJ, Gaines SD (eds) Species invasions: insights into ecology, evolution, and biogeography. Sinauer Sunderland, MA, pp 41–64

Sugiyama A, Vivanco JM, Jayanty SS, Manter DK (2010) Pyrosequencing assessment of soil microbial communities in organic and conventional potato farms. Plant Dis 94:1329–1335

van Bruggena AHC, Semenovb AM (2000) In search of biological indicators for soil health and disease suppression. Appl Soil Ecol 15:13–24

Weller DM, Raaijmakers JM, Gardener BB, Thomashow LS (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu Rev Phytopathol 40:309–348

Weng J, Wang Y, Li J, Shen Q, Zhang R (2013) Enhanced root colonization and biocontrol activity of *Bacillus amyloliquefaciens* SQR9 by *abrB* gene disruption. Appl Microbiol Biotech. doi:10.1007/s00253-012-4572-4

Xu Z, Shao J, Li B, Yan X, Shen Q, Zhang R (2013) Bacillomycin D in *Bacillus amyloliquefaciens* SQR9 contributes to antifungal activity and biofilm formation. Appl Environ Microbiol 79: 808–815

Yao H, Wu F (2010) Soil microbial community structure in cucumber rhizosphere of different resistance cultivars to *Fusarium* wilt. FEMS Microbiol Ecol 72:456–463

Zamioudis C, Pieterse CMJ (2012) Modulation of host immunity by beneficial microbes. Mol Plant Microbe In 25:139–150

Zhang S, Raza W, Yang X, Hu J, Huang Q, Xu Y, Liu X, Ran W, Shen Q (2008) Control of *Fusarium* wilt disease of cucumber plants with the application of a bioorganic fertilizer. Biol Fertil Soils 44:1073–1080

Zhao Q, Dong C, Yang X, Mei X, Ran W, Shen Q, Xu Y (2011) Biocontrol of *Fusarium* wilt disease for *Cucumis melo* melon using bio-organic fertilizer. Appl Soil Ecol 47:67–75

Zhou X, Wu F (2012a) Dynamics of the diversity of fungal and *Fusarium* communities during continuous cropping of cucumber in the greenhouse. FEMS Microbiol Ecol 80:469–478

Zhou X, Wu F (2012b) *p-Coumaric* acid influenced cucumber rhizosphere soil microbial communities and the growth of *Fusarium oxysporum* f.sp. *cucumerinum* Owen. PLoS One 7:e48288

Zhou X, Yu G, Wu F (2012) Responses of soil microbial communities in the rhizosphere of cucumber (*Cucumis sativus* L.) to exogenously applied *p*-hydroxybenzoic acid. J Chem Ecol 38:975–983