Applied Soil Ecology 110 (2017) 146–150

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Short communication

The nutrient preference of plants influences their rhizosphere microbiome

Feng Cai[a,1], Guan Pang[a,1], Youzhi Miao[a], Ruixia Li[a], Rong Li[a], Qirong Shen[a,b], Wei Chen[a,*]

[a] Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, Nanjing Agricultural University, Nanjing 210095, PR China
[b] National Engineering Research Center for Organic-based Fertilizers, Yixing 214200, PR China

ARTICLE INFO

Article history:
Received 17 May 2016
Received in revised form 5 November 2016
Accepted 7 November 2016
Available online 15 November 2016

Keywords:
Tomato
Cucumber
Nutrient preference
MiSeq sequencing
Plant-associated microbiome

ABSTRACT

Many studies in recent decades have shown the signature effect of the host plant in determining the plant-associated microbiome in the soil. However, the important question as to the factors contributing to the selective enrichment of microorganisms in the plant rhizosphere has not been fully addressed. In this study, the role of the nutrient preferences of two plant species, tomato and cucumber, in variations in the soil microbiome were investigated using a five-season continuous pot experiment. The results of MiSeq sequencing showed that these two plants assembled specific bacterial and fungal communities in their rhizospheres, and the soil nutrient status resulting from the plant nutrient preference was identified as a key driver in the development of a plant-specific microbiome.

The plant-associated microbial community in rhizosphere, also referred to as the second genome of the plant, is crucial for plant growth and health (Berendsen et al., 2012; Berg et al., 2014; Mueller and Sachs, 2015). This microbial community is influenced by the physical and chemical properties of the soil (Schreiter et al., 2014; Xun et al., 2015) as well as by the species or even the genotype of the host plant (Chaparro et al., 2012, 2014; Ofek et al., 2014). Recent advances in research on plant-microbe interactions have revealed a clear signature of the host plant in shaping its rhizosphere microbiome, as evidenced by the specific microbial communities hosted by different plant species when grown in the same soil (Ai et al., 2015; Berendsen et al., 2012; Ofek et al., 2014). However, evidence of whether these host-specific communities thriving in the rhizosphere are directly recruited by plant-derived carbons (root exudates) or via preferences for specific soil conditions, such as mineral nutrients and physical structure, is still limited (Berg et al., 2014; Ofek-Lalzar et al., 2014). For instance, in the work of Ai et al. (2015) performed with isotope probing (^{13}C), only a small subset (i.e., Actinobacteria and Proteobacteria) of rhizosphere microbiota was found to be root-feeding communities in wheat. The phyla in that study included members of Acidobacteria, Chloroflexi, Bacteroidetes and Firmicutes that were not directly associated with root exudates, indicating a limited capability of root-derived products to influence the rhizosphere microbiota. Soil factors such as soil pH, C/N ratio, and available P and K are frequently reported to influence the assemblage of microbial communities in different soils (Chaparro et al., 2012; Geisseler and Scow, 2014; Turner et al., 2013; Zhao et al., 2014). Thus, considering the specificity of plant species with respect to the level and types of soil nutrients absorbed, plant nutrient preferences may also play a role in selecting the host-dependent rhizosphere microbiome. However, the shift in soil nutrients resulting from the host plant preference will not appear in the short term. Therefore, to address this question, the rhizosphere microbiomes of two important crops (tomato and cucumber) were determined using high-throughput MiSeq sequencing as well as the soil nutrient properties in a monocropping system with a five-season continuous pot experiment. The aims of this study were (1) to determine whether plant nutrient preference is involved in assembling a host-specific rhizosphere microbiome and (2) to elucidate how plant nutrient preference drives the selection of the rhizosphere microbiome.

The experimental system consisted of growing tomato (*Lycopersicon esculentum* cv. Suhong 2003) and cucumber (*Cucumis sativus* cv. Xinjinyan 4) in pots (31 × 23 cm, diameter × height) at a density of two plants per pot, which were each filled with 10 kg of alfisol (see Table 1 for soil chemical properties). Pot experiments (six pots per species) were conducted continuously five times with repeated and equal mineral fertilizer applications [containing 4 g

* Corresponding author.
E-mail address: chenwei@njau.edu.cn (W. Chen).
[1] These authors contributed to the work equally and should be regarded as co-first authors.

http://dx.doi.org/10.1016/j.apsoil.2016.11.006
0929-1393/

Table 1
Nutrient properties of soil samples.

Soil nutrients	CK	TOM	CUC
NH_4^+-N (mg kg^{-1})	29.31 ± 0.40 a	4.75 ± 0.61 b	5.50 ± 1.64 b
NO_3^--N (mg kg^{-1})	0.79 ± 0.11 c	138.36 ± 1.47 b	287.10 ± 8.84 a
Available P (mg kg^{-1})	99.23 ± 1.48 a	103.10 ± 7.08 a	74.81 ± 0.46 b
Available K (mg kg^{-1})	150.54 ± 3.03 a	122.70 ± 0.37 b	145.02 ± 0.98 a
Available Fe (mg kg^{-1})	46.03 ± 0.08 a	44.98 ± 0.86 a	45.08 ± 0.24 a
Available Mn (mg kg^{-1})	53.71 ± 0.55 b	51.62 ± 0.56 b	58.62 ± 0.86 a
Available Cu (mg kg^{-1})	3.27 ± 0.05 a	3.42 ± 0.01 a	3.23 ± 0.10 a
Available Zn (mg kg^{-1})	10.88 ± 0.14 a	10.38 ± 0.15 ab	10.29 ± 0.13 b
Organic matter (g kg^{-1})	19.22 ± 0.41 a	18.55 ± 0.32 a	18.05 ± 0.87 a
Total N (g kg^{-1})	1.38 ± 0.02 a	1.29 ± 0.00 a	1.39 ± 0.06 a
Total P (g kg^{-1})	1.14 ± 0.00 c	1.91 ± 0.02 a	1.79 ± 0.01 b
Total K (g kg^{-1})	2.77 ± 0.01 c	7.07 ± 0.46 b	8.67 ± 0.03 a
Total Fe (g kg^{-1})	6.49 ± 0.89 b	19.62 ± 0.48 a	21.24 ± 0.09 a
Total Mn (g kg^{-1})	0.32 ± 0.03 b	0.49 ± 0.00 a	0.49 ± 0.00 a
Total Cu (g kg^{-1})	0.01 ± 0.00 b	0.02 ± 0.00 a	0.02 ± 0.00 a
Total Zn (g kg^{-1})	0.08 ± 0.01 b	0.10 ± 0.00 a	0.08 ± 0.00 ab

CK: initial soil sample collected before planting and fertilization; TOM: soil samples collected after five seasons of tomato cultivation; CUC: soil samples collected after five seasons of cucumber cultivation. The mean value ± standard deviation (n = 3). Values with the same letter are not significantly different in a row ($p < 0.05$). NH_4^+-N, ammonium-N; NO_3^- -N, nitrate-N.

plant^{-1} of YaraMila compound fertilizer and 2 g plant^{-1} of YaraLiva-Ca(NO_3)$_2$, both from LEILI Agrochemistry Co., Ltd., Beijing, China] in a greenhouse of the National Engineering Research Centre for Organic-based Fertilizers, Yixing, China. Plants were allowed to grow for 100 days each growing season. Rhizosphere soil samples were collected as described by Hervás et al. (1998) and Zhang et al. (2013) after the fifth growing season. The soil nutrient properties were determined as described in our previous work (Cai et al., 2015). As expected, after five seasons of plant cultivation, the planted soil nutrient concentrations were significantly ($p < 0.05$) changed compared with those of the initial soil sample. For example, ammonium-N had been significantly consumed, whereas nitrate-N and total P, K, Fe, Mn and Zn were significantly accumulated in the later soils. Moreover, despite originating from the same soil, the nutrient status of the soil samples collected from the tomato rhizosphere (TOM) and cucumber rhizosphere (CUC) were significantly different from each other after five replications of monoculture (Table 1). The CUC soil samples showed significantly higher nitrate-N, available K, available Mn and total Fe accumulation and significantly lower total P ($p < 0.05$) than the TOM samples.

Total genomic DNAs were extracted from the soil samples according to the PowerSoil DNA Isolation Kit (MoBio) protocol. PCR reactions (20 μl) were performed in triplicate for each sample, and each reaction volume included 1 × reaction buffer (TAKARA), 0.2 mM dNTPs, 0.1 μM of each primer, 1 U HotStarTaq polymerase (TAKARA), and 2 μl DNA. The thermal cycling conditions were as follows: 2 min initial denaturation at 95 °C; 35 cycles at 94 °C for 20 s, 55 °C for 40 s, and 72 °C for 60 s; and final 2 min extension at 72 °C. Primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′) were used to amplify the V3 V4 hypervariable regions of the 16S rRNA gene. Primers ITS4 (5′-ATCCTCCGCTTATTGATATGC-3′) and ITS3 (5′-GCATCGATGAAGAACGCAGC-3′) were used to amplify the fungal internal transcribed spacer (ITS2) region. Then, the bacterial and fungal microbiomes of the rhizosphere samples were examined with MiSeq sequencing by Genesky Biotechnology Inc. (Shanghai, China). The raw sequence data were processed as described by Huang et al. (2015) and have been deposited in the NCBI database with the accession code SRP070977. The α-diversities for each sample, shown in Table 2 indicated that the TOM had significantly higher fungal diversity (as shown by the higher Chao1, ACE indices and Shannon index) than the CUC after a five-season monoculture, whereas tomato had significantly lower bacterial diversity. The principal coordinate analysis (PCoA, see Supplemental Fig. S1) revealed results consistent with those in Table 2, which suggested that tomato and cucumber had significantly different impacts on the composition of the rhizosphere microbiome. In addition, the dramatic loss of diversity in both rhizospheres can be explained by the effects of monoculture, as monocropping is well known to result in the simplification of the microbial structure (Huang et al., 2013).

A non-parametric test was used to compare the relative abundances of the rhizosphere microbiome of these two plant species (Fig. 1). Specifically, the dominant bacterial phyla Proteobacteria (28%) and Bacteroidetes (26%) and the fungal phyla Basidiomycota (54%) were more highly stimulated in the CUC than in the TOM, in which Actinobacteria (24%) and Ascomycota (54%) were more highly stimulated. The heat map of the top 100 genera within a hierarchical cluster based on Bray-Curtis distance indices showed varying patterns of microbial community structure between the two plants (Fig. 2). The TOM clearly showed a lower evenness of the bacterial community, whereas the CUC showed a lower evenness of the fungal community. The unique plant host signature effect on the rhizosphere microbiome was recently detected in several plants, including *Arabidopsis*, tomato, cucumber, wheat and maize (Lundberg et al., 2012; Ofek et al., 2014; Yuan et al., 2015). However, the mechanism by which plant roots select specific microbes to assemble in the rhizosphere has not been revealed. Ai et al. (2015) suggested that the structure and assembly of the rhizosphere microbiome depended not only on root exudates (which were considered key determinants in several studies, such as Lu et al., 2006; Paterson et al., 2007; Uroz et al., 2010) but also on other cues. However, Ai et al. (2015) did not provide further direct evidence in support of this viewpoint. Our results from Pearson's correlation showed that most of the significant correlations between soil nutrient characteristics and microbial phyla involved the nutrient properties that differed between the two plant species (Table 3). For example, at the genus level, the relative abundance of the *Denitrobacterium* genus was significantly negatively (−0.963) correlated with soil nitrate-N (Supplemental Table S1); that is, the lower accumulation of nitrate-N in the TOM resulted in more *Denitrobacterium* propagation in the soil, as expected. Hence, in the present study, we demonstrated that the nutrient preference of different plants also played a role in shaping their own rhizosphere microbiome,

Table 2
Estimated observed richness and diversity for the different soil samples.

Soil samples[a]	Bacteria			Fungi		
	Chao1	ACE	Shannon	Chao1	ACE	Shannon
TOM	2432 ± 35	2422 ± 32*	5.38 ± 0.24*	1618 ± 70*	1604 ± 61**	4.29 ± 0.15**
CUC	2342 ± 49	2315 ± 38	5.80 ± 0.02	1430 ± 24	1415 ± 30	3.27 ± 0.25

Values are the mean ± standard deviation (n = 3).Values of *$p < 0.05$ and **$p < 0.01$ were considered to represent statistically significant differences between the two plant species. Chao1, richness of the Chao1 estimator; operational taxonomic units; ACE, abundance-based coverage estimator; Shannon, nonparametric Shannon diversity index.
[a] TOM: rhizosphere soil collected from tomato roots; CUC: rhizosphere soil collected from cucumber roots.