Stable Microbial Community and Specific Beneficial Taxa Associated with Natural Healthy Banana Rhizosphere^S

Lin Fu^{1,2}, Yannan Ou², Zongzhuan Shen², Beibei Wang³, Rong Li^{2*}, and Qirong Shen²

¹Institute of Environmental Processes and Pollution Control, and School of Environment and Civil Engineering, Jiangnan University, Wuxi, 214122, P.R. China

²Jiangsu Provincial Key Lab of Solid Organic Waste Utilization, Jiangsu Collaborative Innovation Center of Solid Organic Wastes, Educational Ministry Engineering Center of Resource-saving Fertilizers, Nanjing Agricultural University, Nanjing, 210095, P.R. China

³Hainan Key Laboratory for Sustainable Utilization of Tropical Bio-resources, Institute of Tropical Agriculture and Forestry, Hainan University, Haikou 570228, P.R. China

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*Corresponding author
Phone: +86-02584395535
Fax: +86-02584395535
E-mail: lirong@njau.edu.cn

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Banana planting altered microbial communities and induced the enrichment of *Fusarium oxysporum* in rhizosphere compared with that of forest soil. Diseased plant rhizosphere soil (WR) harbored increased pathogen abundance and showed distinct microbial structures from healthy plant rhizosphere soil (HR). The enriched taxon of *Bordetella* and key taxon of *Chaetomium* together with some other taxa showed negative associations with pathogen in HR, indicating their importance in pathogen inhibition. Furthermore, a more stable microbiota was observed in HR than in WR. Taken together, the lower pathogen abundance, specific beneficial microbial taxa and stable microbiota contributed to disease suppression.

Keywords: *Fusarium* wilt, rhizosphere microbiota, stability, co-occurrence

Banana, the world's favorite fruit crop, is now suffering a devastating soilborne disease caused by *Fusarium oxysporum* f. sp. *cubense* race 4 (FOC4), which severely hinders banana production worldwide [1, 2]. Since the pathogen can survive in soil as chlamydospores for decades, it is very difficult to eradicate [3]. Recent studies have highlighted the ability of rhizosphere microbiomes to suppress plant disease, mainly mediated by competition, antibiosis, plant growth promotion or systemic induced resistance [4]. Thus, an improved understanding of the changes in rhizosphere microbial ecology associated with healthy and diseased banana plants *in natura* could facilitate the development of efficient biological control strategies for improving plant growth and health. However, the difference in the rhizosphere microbiota between healthy and diseased banana plants is still poorly understood.

In this study, we used Illumina MiSeq sequencing and

quantitative PCR (qPCR) to decipher the rhizosphere bacterial and fungal microbiota associated with healthy and diseased *Cavendish* bananas in newly converted banana orchards continuously planted with bananas for two years in Chengmai County, Hainan Province, China. The roots from healthy and diseased banana plants and the adjacent forest soil before planting were collected in September 2013 before banana harvesting. The selected diseased plants infected with FOC4 showed similar disease severity. In total, 10 healthy (HR) and 10 diseased (WR) banana root composite samples and 10 original forest soil (FS) samples were collected. Rhizosphere soil was obtained as previously described [5]. Total genomic DNA was isolated from soils using the PowerSoil DNA Isolation Kit (Mbio Laboratories, USA), following the manufacturer's instructions. The abundances of bacteria and fungi were quantified using qPCR [6] (see the Supplementary Information for additional

details). The V4 region of the bacterial 16S rRNA gene and fungal first internal transcribed spacer region (ITS1) were amplified from the soil genomic DNA by the primers 515F/806R [7] and ITS1F/ITS2 [8], respectively. The PCR amplification conditions and the protocol for library construction were performed as previously described [9]. All constructed libraries were sequenced on an Illumina MiSeq sequencing platform at Novogene Bioinformatics Institute (China). The sequence data of the bacteria and fungi are available at the accession number PRJNA533861 in the NCBI Sequence Read Archive database. The raw sequences obtained were analyzed as previously described [3] with minor modifications (see the Supplementary Information for additional details).

No significant difference was observed between healthy (HR) and diseased (WR) banana rhizosphere soil for either the bacterial or fungal diversity index (Fig. S1). Frequently, increased microbial rhizosphere diversity is correlated with a low incidence of pathogen outbreaks [10], which was also proposed to counteract *Fusarium* wilt in banana [3]. In contrast, the results observed in this study indicate that the higher rhizosphere microbial diversity may be advantageous but not essential for the plant-protective microbiome. Moreover, the microbial communities of HR were markedly more stable in terms of diversity than those of WR, which was supported by the lower coefficient of variability (CV) values in HR. These results showed that lower variability was associated with healthy rhizosphere

microbiota.

Principal coordinate analysis (PCoA) based on the Bray-Curtis distance metric displayed significant differences in the bacterial ($F_{\text{PERMANOVA}} = 18.4$, $R^2 = 0.57$, $p = 0.001$) and fungal ($F_{\text{PERMANOVA}} = 19.21$, $R^2 = 0.59$, $p = 0.001$) community structure among all soil samples (Figs. 1 and S2). These results reveal that banana planting can significantly alter the soil microbiome (FS), which is in accordance with previous studies [11] and that plant health status is also the driver of variation in soil microbial β -diversity. The fact that the root microbiota of healthy plants differ from that of diseased plants is well documented for various crops, such as wheat [12] and citrus [13]. Similar to the alpha diversity index, the rhizosphere microbiota of healthy banana plants also showed stable community structure with low CV values, while the rhizosphere microbiota of diseased banana plants exhibited high CV values, suggesting large variability in community structure. Likewise, Köberl *et al.* also found that rhizospheric *Gammaproteobacteria* communities of pathogen-infected plants showed higher variability than those in healthy plants in Costa Rica [14]. Functional reliability or stability in terms of declining variability was used as a fundamental ecosystem parameter [15, 16], and high CV values generally exhibit low ecosystem stability [17]. Furthermore, the decrease in community variability was correlated with the increase in invasion resistance [16, 18], such that Wei *et al.* suggested that communities with stabilizing configurations can reduce pathogen invasion

Fig. 1. Principal coordinate analysis (PCoA) of (A) bacterial and (B) fungal community structure based on the Bray-Curtis distance metric among all soil samples.

WR, rhizosphere soil of diseased banana plants; HR, rhizosphere soil of healthy banana plants; FS, forest soil. The coefficient of variability (CV, standard deviation divided by the mean) in community structure based on Bray-Curtis distance within the group was also calculated and shown.

Fig. 2. (A) Histogram of the LDA scores computed for differentially abundant fungal and bacterial genera between rhizosphere soil of healthy and diseased banana plants. (B) Estimated absolute abundance (EAA) of *Fusarium* and FOC4 (fungal Seq2, most closely related to FOC4, Fig. S4) based on qPCR and Illumina MiSeq sequencing in all soil samples. The bottoms and tops of boxes represent the 25 and 75% quartiles, respectively. The horizontal bars and diamond within boxes represent the median and mean, respectively. WR, rhizosphere soil of diseased banana plants; HR, rhizosphere soil of healthy banana plants; FS, forest soil.

success [19]. Consequently, stable rhizosphere microbiota with low variability may facilitate resistance to disturbance, make the overall community more functional, and benefit plant disease resistance.

The composition of the soil microbial community was investigated by LEfSe analysis (Figs. 2A and S3). The rhizosphere soil of healthy banana plants enriched the genera *Streptacidiphilus*, *Acidobacteria_GP1_NA*, *Salmonella*, *Leifsonia*, *Noviherbaspirillum*, *Pantoea*, *Frateruia*, *Bordetella*, *Kosakonia*, *Marmoricola*, *Enterobacter*, *Fulvimonas* and *Sporidiobolales_unclassified* and depleted *Humibacter*, *Gibellulopsis*, *Purpureocillium*, *Bisifusarium*, *Phaeohelotium*,

and *Codinaeopsis* compared with the WR samples (Fig. 2A). Although no significant difference of *Fusarium* abundance was observed between HR and WR, a significantly higher abundance of pathogen FOC4 was observed in WR than in HR (Wilcoxon *t*-test, $p = 0.01$) (Fig. 2B). This finding is in accordance with previous findings that *Fusarium* wilt-infected soil contained more pathogen than healthy soils [20]. In addition, the abundances of pathogen in both HR and WR were significantly higher than that in the original forest soil ($p < 0.001$) (Fig. 2B), demonstrating that banana cultivation induced the enrichment of *F. oxysporum* [21].

To understand the potential microbes involved in disease

Fig. 3. (A) Z_i - P_i plot showing the distribution of nodes based on their topological roles. Each symbol represents a node in HR (pink) and WR (green) network. The threshold values of Z_i (within-module connectivity) and P_i (among-module connectivity) for categorizing nodes were 2.5 and 0.62, respectively. The module hubs and connectors are labeled with sequence numbers and classified annotations. (B) The co-occurrence network between pathogen (fSeq2) and other microbial taxa in HR samples. Nodes correspond to ASVs, and connecting edges demonstrate correlations between them. The green edges indicate positive interactions between two microbial nodes, while red edges indicate negative interactions. HR, rhizosphere soil of healthy banana plants; WR, rhizosphere soil of wilt banana plants.

suppression, a co-occurrence network has been generated using SparCC approach. Based on the topological roles of nodes, four keystone taxa were found in HR, whereas none was observed in WR (Fig. 3A). These results suggested that more functional taxa, which played vital roles in maintaining microbial network structure and function [22], were involved in the HR co-occurrence pattern. Then, we focused on all negative links with pathogen FOC4 in HR. In total, 10 significant negative correlations ($p < 0.05$) were found between pathogen and some taxa, among which enriched taxon of *Bordetella* and keystone taxon of *Chaetomium* were shown (Fig. 3B). Although no direct suppression related to *Fusarium* was reported, *Bordetella*, a member of *Burkholderiales*, was reported to produce the siderophore of Alcaligin [23] and show antagonistic activity against phytopathogenic fungus [24]. *Chaetomium* was reported as a broad spectrum biological fungicide for plant disease control, including *Fusarium* wilt [25]. In addition, some other genera also negatively correlated to pathogen in HR (grey circle in Fig. 3B) were observed. Considering that these taxa were not enriched or acted as keystone taxa in the network, we speculate that *Bordetella* and *Chaetomium* should play vital roles in pathogen inhibition. Also, the presence of a low amount of pathogen

in the healthy plant rhizosphere may be attributed to the collective actions of the resident microbial community [26]. Taken together, these findings indicate that not only the absolute abundance of pathogen but also other microbial taxa interactions and community stability, determine the significant difference in plant health status.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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