Inducing Banana Fusarium Wilt Disease Suppression through Soil Microbiome Reshaping by Pineapple-Banana Rotation Combined with Biofertilizer Application

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Abstract

Crop rotation and biofertilizer application have historically been employed as efficient management strategies for soil-borne disease suppression through soil microbiome manipulation. However, how this occurs and to what extent the combination of methods affects the microbiota reconstruction of diseased soil is unknown. In this study, pineapple-banana rotation combined with biofertilizer application was used to suppress banana Fusarium wilt disease, and the effects on both bacterial and fungal communities were investigated using the MiSeq Illumine sequencing platform. Our results showed that pineapple-banana rotation significantly reduced Fusarium wilt disease incidence and the application of biofertilizer caused additional suppression. Bacterial and fungal communities thrived using rotation combined with biofertilizer application: taxonomic and phylogenetic α-diversity of both bacteria and fungi increased along with disease suppression. Between the two strategies, biofertilizer application predominantly affected both bacterial and fungal community composition compared to rotation. *Burkholderia* genus may have been attributed to the general wilt suppression for its change in network structure and high relative importance in linear models. Our results indicated that pineapple-banana rotation combined with biofertilizer application has strong potential for the sustainable management of banana Fusarium wilt disease.

Keywords: Banana Fusarium wilt; Pineapple-banana rotation; Biofertilizer; Disease suppression; Microbiome structure

1 Introduction

Banana Fusarium wilt disease caused by *Fusarium oxysporum* f. sp. *cubense* (FOC) race 4 forms a major constraint on the yield and quality of banana production (Ploetz, 2015; Butler, 2013). Multiple studies have revealed that individual measures, such as fumigation (Duniway, 2003; Liu et al., 2016), chemical fungicides (Nel et al., 2007), crop rotation (Zhang et al., 2013b), and bio-control (Wang et al., 2013) have particular effects on reducing the incidence of soil-borne diseases by disrupting soil microbial community membership and structure. Traditionally, fumigation, chemical fungicides, or crop rotation is used in fields with high incidence rates, and bio-control is used in low- or new-incidence fields because of its apparent mild effect (Shen et al., 2018). However, single measures often have limited effectiveness, and a few studies regarding soil-borne disease suppression focused on using multiple strategies to improve control efficiency. Like Shen et al. (2018) reported that biofertilizer application after fumigation with lime and ammonium bicarbonate was an effective strategy to control banana Fusarium wilt disease. Thus, while many measures can individually slow down the spread of Fusarium wilt disease (Pda et al., 2017), control effects can be accelerated and amplified by using more than one agricultural practice.

Among the management strategies, chemical pesticides are optimally effective against soil-borne plant pathogens, but this strategy is environmentally hazardous, and not only causes soil and water pollution but also induces the emergence of drug-resistant strains (Le et al., 2016). Biological control using beneficial soil microorganisms such as *Bacillus* and *Trichoderma* against soil-borne pathogens is considered as a sustainable alternative to chemical pesticides (Alabouvette et al., 2009; Fravel et al., 2003; Qiu et al., 2012). Biofertilizers combine the advantages of introducing beneficial microbes with organic material that not only occupy niches but also create additional niches for beneficial indigenous microbes (Cai et al., 2017;

Zhang et al., 2013). In our previous study, we developed a biofertilizer containing the *Bacillus* strain isolated from the rhizosphere of a continuously cropped banana that promoted plant growth and suppressed the banana Fusarium wilt (Shen et al., 2015; Fu et al., 2016; Fu et al., 2017). Therefore, biofertilizer application is a practicable and worthy measure for banana Fusarium wilt suppression.

In addition, crop rotation is also considered a highly efficient and environmentally friendly alternative method in soil-borne disease control (Krupinsky et al., 2002). Crop rotation breaks the microflora and chemical characteristics of continuously mono-cropped soil leading to the control of soil-borne diseases (Christen and Sieling, 2010; Yin et al., 2010). The mechanisms of soil-borne disease suppression induced by crop rotation include inhibition of pathogen reproduction through allelochemical secretion, stimulation of antagonistic microbes against pathogens, and improvement of rhizosphere microbial community structure by introducing different carbon compounds into the soil through root exudates or residues (Robert et al., 2014). In our previous study, besides biofertilizer application, our work also showed that the banana-pineapple rotation efficiently suppressed the banana Fusarium wilt disease (Wang et al., 2015). However, the combined control efficiency of the two measures (pineapple-banana rotation plus biofertilizer application) remains unknown. Thus, there is a great need to explore efficient disease suppressing-combined approaches for the banana Fusarium wilt control and to progress towards maintaining sustainable worldwide industrial banana development.

The occurrence of soil-borne disease is mainly due to the imbalance of soil microbial communities caused by soil-borne pathogen blooms (Mendes et al., 2014). Effective soil-borne disease suppression management strategies must demonstrate significant changes in the soil microbial community in addition to pathogen minimization (Cha et al., 2016; Chaparro et al., 2012; Gerbore et al., 2014; Mazzola

and Freilich, 2017). Our previous reports proved the effectiveness of microbial agents for biocontrol by changing the structure of soil microbial communities (Fu et al., 2017; Shen et al., 2015). We also investigated the influences of quarterly rotation (pineapple) on *Fusarium* population density and soil microbial community structure while attempting to explore the mechanisms of pineapple-banana rotation on soil borne-disease suppression (Wang et al., 2015). Our results suggested that fungal community structure and several genera introduced in the rotation season may have been the most critical factors in decreasing soil *Fusarium* population.

Unlike intercropping, controlling *Fusarium* pathogen accumulation through effective crop rotation should be maintained for at least two seasons, including rotation and a subsequent season (Bullock, 1992; Lupwayi et al., 1998). The pineapple and banana growth cycles in our rotation pattern require long durations (almost 15 and 10 months, respectively, in Hainan Province, China). Thus, the soil microbial community structure of the original season is very important in evaluating rotation validity. Furthermore, how the soil microbial community structure changes using the combined control efficiencies of the two measures (pineapple-banana rotation and biofertilizer application) remains unknown.

We hypothesized that Fusarium wilt can be effectively controlled in high-disease incidence fields by pineapple-banana rotation and that the control efficiency can be improved by adding biocontrol to the rotation. In addition, this scheme will concurrently change the soil microbial community membership and structure. Therefore, based on our previous research, we conducted field experiments to investigate the effects of pineapple-banana rotation combined with biofertilizer on next season banana Fusarium wilt disease suppression and soil microbial communities. Our objectives were to 1) determine the direct effects of pineapple-banana rotation alone and pineapple-banana rotation combined with biofertilizer application to control

banana Fusarium wilt disease; 2) explore the characteristics of the soil microbial communities prompted by crop rotation and biocontrol strategies after banana harvest using the MiSeq platform, and 3) evaluate the probable disease suppression mechanisms caused by rotation and biocontrol strategy.

2 Materials and Methods

2.1 Field experimental design

The field experiment was set up at the site of Hainan Wanzhong Industrial Co., Ltd., China, a company that specialized in banana planting during December 2011 to June 2014. The field soil had a chemical background of pH 5.12, soil organic matter (SOM) 5.57 g kg^{-1}, NH_4^+-N 7.39 mg kg^{-1}, NO_3^--N 6.68 mg kg^{-1}, available P 56.9 mg kg^{-1} and available K 176.4 mg kg^{-1}. The organic fertilizer (OF) used in our study was supplied by Lianye Biofertilizer Engineering Center, Ltd., Jiangsu, China, which was the first fermentation of amino acid fertilizer and pig manure with a 2:3 weight ratio, respectively. The biofertilizer (BIO) was a secondary fermentation based on OF according to the solid fermentation method (Wang et al., 2013). The research was carried out in a field in which a serious Fusarium wilt disease incidence (> 50%) was observed after continuous banana cropping for 6 years. Nine replicates were set up in each treatment with a randomized complete block design, and the area of each block was 300 m^2. Banana cultivar *Musa acuminate AAA Cavendish cv*. Brazil and the pineapple cultivar Golden pineapple were used in the field experiment. Three treatments were assigned: (1) banana continuously cropped for two years with common organic fertilizer application (BOF); (2) banana planted after an eighteen-month pineapple rotation with common organic fertilizer application in the banana season (POF); and (3) banana planted after an eighteen-month pineapple rotation treatment with biofertilizer application (PBIO). In the rotation system, pineapple and

152 banana were planted at the densities of 45000 and 2,400 seedlings ha^{-1}, respectively.
153 All organic fertilizer was applied to the soil at once as base fertilizer before banana
154 planting. Other measures were consistent with common banana production.

155 **2.2 Banana Fusarium wilt disease incidence statistics**

156 Old leaves yellowing, stem crack and new leaves diminishing were the three typical
157 wilt symptoms of banana Fusarium wilt disease. Disease incidence was calculated
158 based on the appearance of all three symptoms weekly since the first sick banana
159 plant appeared. Finally, banana wilt disease incidence was determined at the harvest
160 time. The percentage of sick plants among the total banana plants was calculated as
161 the Fusarium wilt disease incidence.

162 **2.3 Soil sample collection and DNA extraction**

163 During the harvest time of last banana planting season, five healthy plants were
164 randomly picked in each biological replicate plot for soil sampling. Soil samples were
165 collected from four random sites around the banana plant at 10 cm distance, and a soil
166 column was picked out at the depth of 20 cm using a soil borer at each sampling site.
167 All five soil columns from each biological replicate plot were mixed for DNA
168 extraction. All mixed samples were placed in cold storage and transported to the
169 laboratory. After passing soil through a 2-mm sieve, total soil DNA was extracted
170 using Clean Soil DNA Isolation Kits (MoBio Laboratories Inc., Carlsbad, USA) from
171 fine-grained soil. After the determination of DNA concentration and quality using
172 NanoDrop 2000 (Thermo Scientific, USA), soil DNA was diluted to a concentration
173 of 20 ng μl^{-1} for PCR amplification.

174 **2.4 Polymerase chain reaction amplification and Illumina Miseq sequencing**

175 Primers F520 (5'-AYTGGGYDTAAAGNG-3') and R802 (5'-
176 TACNVGGGTATCTAATCC-3') were chosen to amplify the V4 regions of 16 S

177 rRNA gene (Claesson et al., 2009). Primers ITS (5'-GGA AGT AAA AGT CGT
178 AAC AAG G-3') and ITS (5'-TCC TCC GCT TAT TGA TAT GC-3') were chosen
179 for amplification of the fungal ITS region (Schoch et al., 2012).

180 PCR reactions for each sample were performed according to the established
181 protocols of Xiong et al. (2016). A total of 27 cycles were performed to amplify the
182 templates. After purification, PCR products were diluted to a concentration of 10 ng
183 μl^{-1}. Fungal and bacterial PCR products sequencing were performed on the Illumina
184 MiSeq platform of Personal Biological Co., Ltd (Shanghai, China).

185 **2.5 Bioinformatic analysis**

186 Raw sequences were separated based on the unique 6-bp barcode and sheared of the
187 adaptor and primer using QIIME (Caporaso et al., 2010). Forward and reverse
188 sequences were merged after the removal of low-quality sequences. Then, the merged
189 sequences were processed to build the operational taxonomic unit (OTU) at an
190 identity level of 97% according to the UPARSE pipeline. Next, representative
191 sequences of each OTU were classified in the RDP and UNITE databases for bacteria
192 and fungi, respectively (Edgar, 2013; Wang et al., 2007). All raw sequences were
193 deposited in NCBI under the accession number SRP234066.

194 To compare the relative levels of OTU diversity across all samples, a rarefaction
195 curve was formed using Mothur software (Schloss et al., 2009). The fungal and
196 bacterial diversity was estimated using phylogenetic diversity (PD) indices and Chao1
197 richness, which were also calculated based on neighbor-joining phylogenetic trees
198 generated using Mothur pipeline (Faith, 1992).

199 To compare bacterial and fungal community structures among all soil samples,
200 principal coordinate analysis (PCoA) was set up based on the unweighted UniFrac
201 metric matrix (Lozupone et al., 2005). Multiple regression tree (MRT), based on

Bray-Curtis distance metric, was carried out to evaluate the effects of rotation and fertilizer type on the whole soil bacterial and fungal community by using vegan and MVPART wrap package in R (version 3.2.0). In addition, to exclude the influence of low abundance species, only the OTUs with the average relative abundance of equal or greater than 0.1% in each sample were retained (defined as retained OTUs).

2.6 Network analyses

Based on retained OTUs, interaction networks between OTUs were constructed using the phylogenetic Molecular Ecological Network (pMEN) method according to Zhou et al. (2011) and Deng et al. (2012). All analyses were performed using the Molecular Ecological Network Analyses Pipeline (MENA). Cytoscape 2.8.2 software was used to visualize the network.

2.7 Statistical analysis

Statistical difference analysis amomg three treatments was carried out using SPSS 20.0 and R software. Pearson correlations among disease incidence, different Phylum and *Fusarium*-relative abundance were analyzed in R. Linear models analysis was performed using R after stepwise model selection considering Akaike information criteria.

3 Results

3.1 Disease incidence and relative abundance of *Fusarium*

Pineapple rotation and biofertilizer application effectively reduced the Fusarium wilt disease incidence and the relative abundance of *Fusarium* in the next season's banana plantation (**Fig. 1A and B**). The incidence of banana Fusarium wilt in the POF and PBIO treatments was 33.3% and 12.3%, respectively, which was significantly lower than that in the BOF treatment (66.8%). PBIO treatment of rotation and biofertilizer

application showed the lowest disease incidence with a 63.1% decrease compared to POF (**Fig. 1B and Table S1**). The relative abundance of *Fusarium* showed the same tendency with disease incidence, so the relative abundance of *Fusarium* and disease incidence were significantly correlated as revealed by MiSeq sequencing data (**Fig. 1C**).

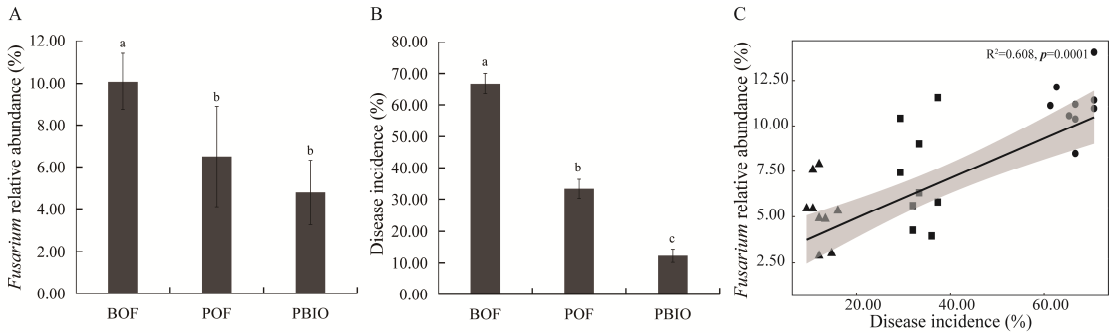

Figure 1.Relative abundance of *Fusarium* (A), Fusarium wilt disease incidence (B) and Pearson correlations between Fusarium wilt disease incidence and *Fusarium* relative abundance (C). BOF = banana continuously cropped with OF application; POF = banana-pineapple rotation with OF application in the banana season; and PBIO = banana-pineapple rotation with BIO application in the banana season. Bars above the histogram represent standard errors and different letters indicate significant differences ($p < 0.05$) according to multivariate variance analysis and multiple comparison results.

3.2 General analyses of the high-throughput sequencing data

After quality control, 908,506 *16S rRNA* and 1,950,262 ITS sequences were retained and based on 97% similarity, a total of 8,346 *16S* and 5,647 ITS operational taxonomic units (OTUs) were obtained. For bacteria, Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria and Verrucomicrobia were the most abundant phyla with >1% relative abundances. For fungi, Ascomycota, followed by Basidiomycota, Chytridiomycota, Zycomycota, and Glomeromycota were the abundant phyla (**Fig. 2**). ANOVA showed that Chlamydiae, Cyanbacteria/chloroplast,

Gemmatimonadetas, Nitrospirae, Planctmycetes, and Verrucomicrobia abundances were significantly higher in the PBIO and POF treatment samples than those in the BOF treatment, and the relative abundance of Ascomycota was lower in the PBIO treatment (Duncan test, $p < 0.05$).

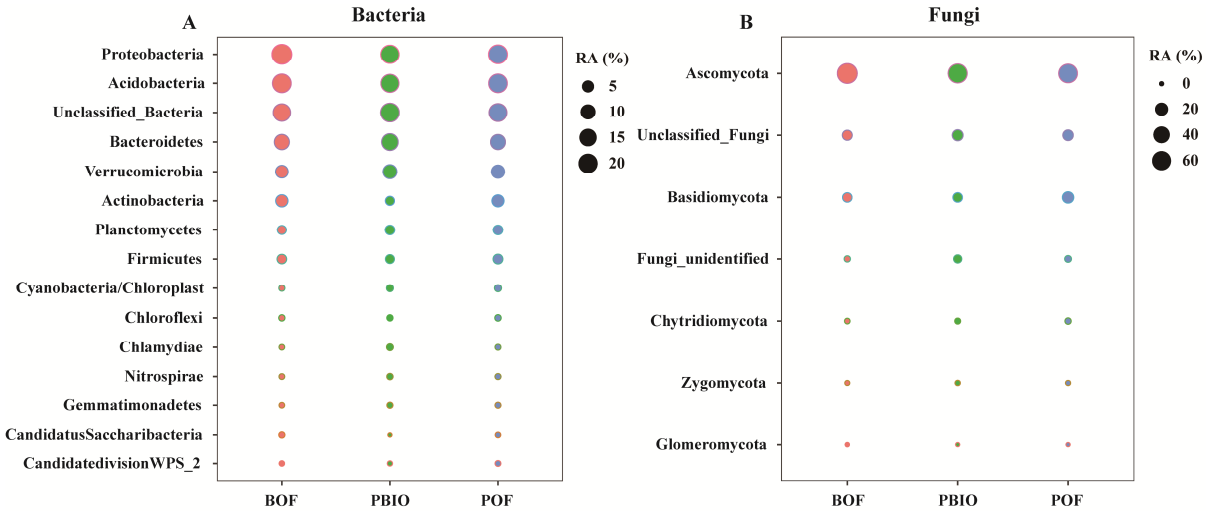

Figure 2. Bubble chart of bacterial (A) and fungal (B) phyla in BOF, POF and PBIO treatments. BOF = banana continuously cropped with OF application; POF = banana-pineapple rotation with OF application in the banana season; and PBIO = banana-pineapple rotation with BIO application in the banana season; Values represent the average abundance across the nine replicate libraries for soil samples collected from each treatment.

3.3 Effect of pineapple rotation and biofertilizer application on soil microbial diversity and community structure

Rarefaction analyses, Chao1 and Faith's PD were performed to characterize α-diversity. Rarefaction analyses showed that the number of OTUs tended to smooth at 14,900 selected bacterial sequences and 34,943 fungal sequences. Compared to BOF treatment, more OTUs were observed in POF and PBIO treatments, both for bacteria and fungi, and the PBIO treatment exhibited the highest value of all treatments (**Table**

1, **Fig. S1**). Compared to BOF treatment, the pineapple-banana rotation treatments, POF and PBIO, increased both taxonomic and phylogenetic α-diversity of both bacteria and fungi. In addition, PBIO treatment showed the highest Chao1 richness and Faith's PD values (**Table 1**).

Table 1. Bacterial and fungal α-diversity indexes of the three treatments. BOF = banana continuously cropped with OF application; POF = banana-pineapple rotation with OF application in the banana season; PBIO = banana-pineapple rotation with BIO application in the banana season; Values represent the average index of nine replicates. Means followed by different letters for a given factor are significantly different ($p < 0.05$; Duncan test).

	Treatment	Numbers of Otus	Chao1	Faith's PD
Bacteria	BOF	2606±71b	3906.81±275.21 b	48.47±1.51 b
	POF	2963±613 a	4444.28±189.98 a	51.61±0.87 a
	PBIO	3210±108 a	4751.95±149.49 a	52.10±1.11 a
Fungi	BOF	1163±64 b	1751.71±74.85 a	114.88±4.78 b
	POF	1277±708 ab	1705.78±126.73 a	120.24±2.12 b
	PBIO	1496±980 a	2096.32±323.60 a	127.31±7.91 a

We evaluated microbial community structure by using PCoA based on a UniFrac unweighted distance matrix to analyze differences in community composition of three treatments. Fungal PCoA showed three distinct groups representing samples taken from three treatments; however, bacterial PCoA showed only two groups. Unweighted UniFrac distances showed that PBIO treatment was separate from BOF and POF treatments along the first component (PCoA1) both in bacteria and fungi. POF treatment was separated from BOF treatment along the second component in fungi, whereas in bacteria, POF and BOF treatments were not separate along the second component (**Fig. 3A**).

Furthermore, MRT results indicated that biofertilizer application had the largest deterministic influence on the composition of both bacterial and fungal communities, and cultivation was secondarily important. Driven by fertilization, PBIO treatment was separated from BOF and POF treatments, and then BOF and POF treatments were driven by cultivation (**Fig. 3B**).

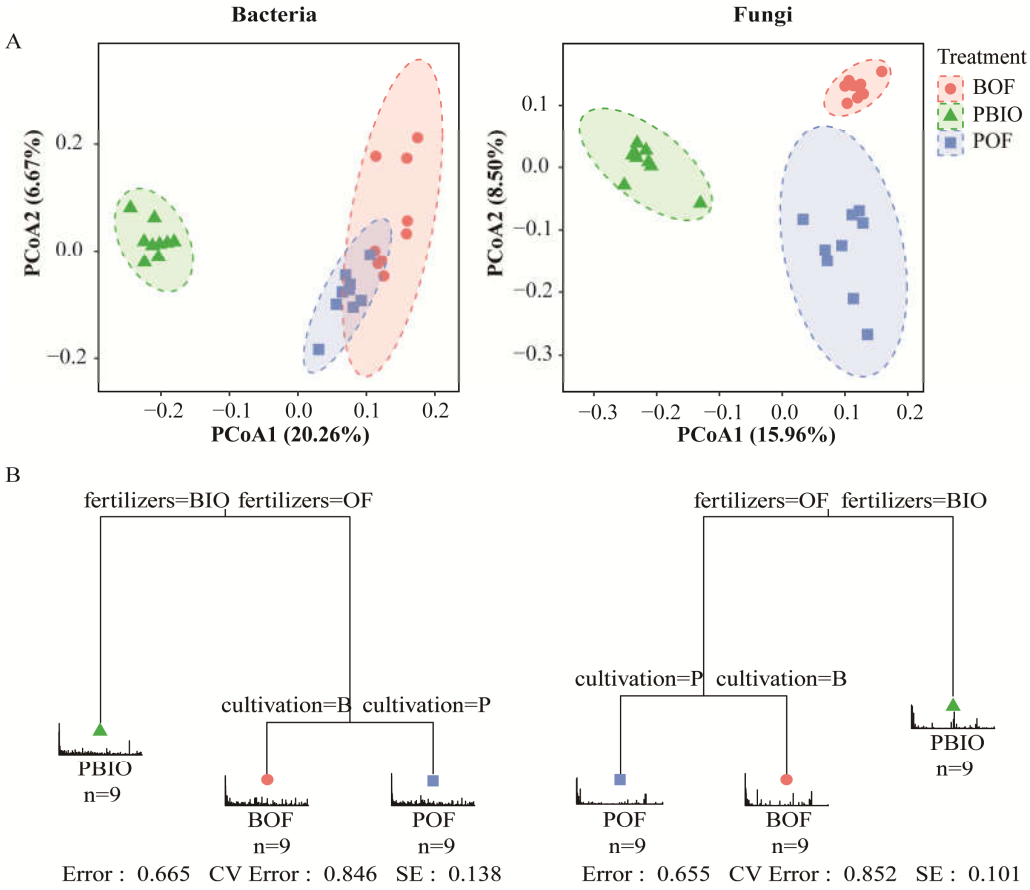

Figure 3. (A) UniFrac-unweighted principal coordinate analysis of fungal and bacterial community structures in different treatments. (B) Multiple regression tree (MRT) analysis for the bacterial and fungal communities showed the variables of fertilization and cultivation in each branch. BOF = banana continuously cropped with OF application; POF = banana-pineapple rotation with OF application in the banana season; PBIO = banana-pineapple rotation with BIO application in the banana season.

3.4 Effect of pineapple rotation and biofertilizer application on soil fungal and bacterial community composition

The results of the phyla correlation with *Fusarium* abundance showed that seven bacterial phyla and three fungal phyla were significantly correlated with pathogen abundance (**Tables S3 and S4**). Moreover, the correlation of fungi was significantly higher with *Fusarium* abundance compared to bacteria based on the percentage of *Fusarium*-related phyla (**Fig. 4B**).

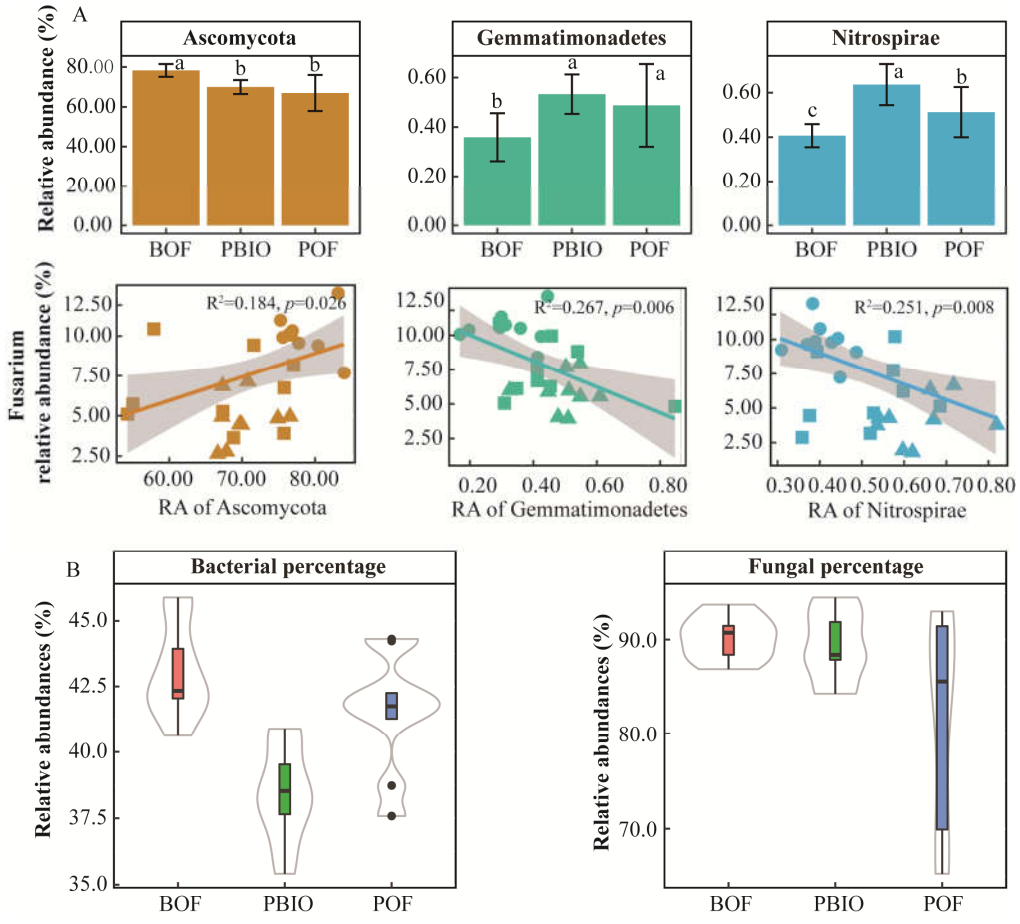

Figure 4. Relative abundance of key phyla and linear regression relationship between key phyla and disease incidence (A). Percentage of *Fusarium* related bacterial and fungal phyla in all treatments (B). BOF = banana continuously cropped with OF application; POF = banana-pineapple rotation with OF application in the banana season; PBIO = banana-pineapple rotation with BIO application in the banana season. Different letters above the bars indicate a significant difference at the 0.05 probability level according to the Duncan test.

3.5 Key topological properties of the networks

We built networks to show interactions among genera in different treatments using OTUs with a relative abundance greater than 0.1%. A total of 301 OTUs were selected from the BOF treatment (122 bacterial and 179 fungal), 323 OTUs were selected from the PBIO treatment (152 bacterial and 171 fungal), and 324 OTUs were selected from the POF treatment (140 bacterial and 184 fungal). Random matrix theory was used to build the networks. As shown in **Fig. 5**, each node represents an OTU, each link shows a significant correlation between two OTUs, red and green represent bacterial and fungal OTUs, respectively, and blue and red represent positive and negative correlations, respectively.

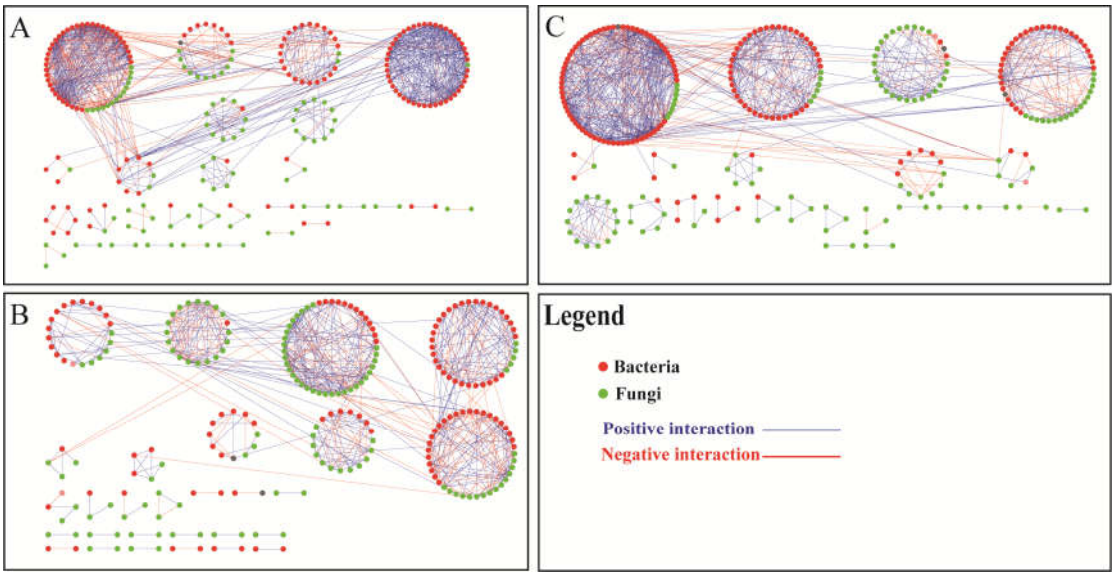

Figure 5. Network plots of bacterial and fungal communities in soil BOF (A), PBIO (B) and POF(C). BOF = banana continuously cropped with OF application; POF = banana-pineapple rotation with OF application in the banana season; PBIO = banana-pineapple rotation with BIO application in the banana season; Red nodes indicate bacteria; Green nodes indicate fungi; red lines between nodes (links) indicate negative interaction; and blue lines indicate positive interaction.

Networks with 286 (143 bacterial and 98 fungal), 245 (122 bacterial and 123 fungal), and 241 (163 bacterial and 123 fungal) nodes were selected from the BOF,

PBIO, and POF treatments, respectively. The F/B ratios that represent the ratio of fungal to bacterial nodes were 0.69, 1.01, and 0.75 in the BOF, PBIO, and POF treatments, respectively. These results suggest more active fungal OTUs in the PBIO treatment followed by the POF and BOF treatments.

The structure index network from different treatments showed 24, 28, and 30 modules in the BOF, PBIO, and POF treatments, respectively (**Table 2**).

Table 2. Topological properties of the empirical and associated random pMENs of microbial communities under BOF, POF and PBIO. BOF = banana continuously cropped with OF application; POF = banana-pineapple rotation with OF application in the banana season; PBIO = banana-pineapple rotation with BIO application in the banana season; Avg K = average connectivity; Avg CC = average clustering coefficient; and GD = average path distance.

			Empirical networks				Random networks		
Treatment	Network size	R^2	AvgK	AvgCC	GD	Modularity	AvgCC	GD	Modularity
BOF	241	0.793	6.71	0.366	2.921	0.62（30）	0.073	2.96	0.322
POF	286	0.796	5.64	0.412	3.739	0.64（24）	0.041	3.34	0.385
PBIO	245	0.739	5.16	0.397	3.642	0.72（28）	0.033	3.28	0.407

The threshold value *Zi* measures the connected degree between two nodes in the same module, and *Pi* measures the connected degree between two nodes from different modules. According to the *Zi* and *Pi* values found in our study, all nodes were divided into four categories (**Fig. 6**). Three nodes, two from the PBIO network and one from the POF network, were categorized as generalists (module hubs) with intense connectivity to many nodes in the same modules. However, there was no module hub found in the BOF network. Fourteen nodes were categorized as connectors (generalists) with high connectivity to several modules, eight from the

BOF network and six from the PBIO network. Interestingly, module hubs (generalists) were only found in the pineapple-banana treatments (PBIO and POF), and connectors (generalists) and module hubs (generalists) were found at the same time only in the pineapple-banana with the biofertilizer applied treatment (PBIO). Annotation information from all generalists showed that bacterial OTU2 and OTU3013 belonging to *Burkholderia* were generalists in the PBIO network but were absent in the POF and BOF networks. Additionally, another generalist OTU4869, from the PBIO network was identified as *Gp6* in Acidobacteria.

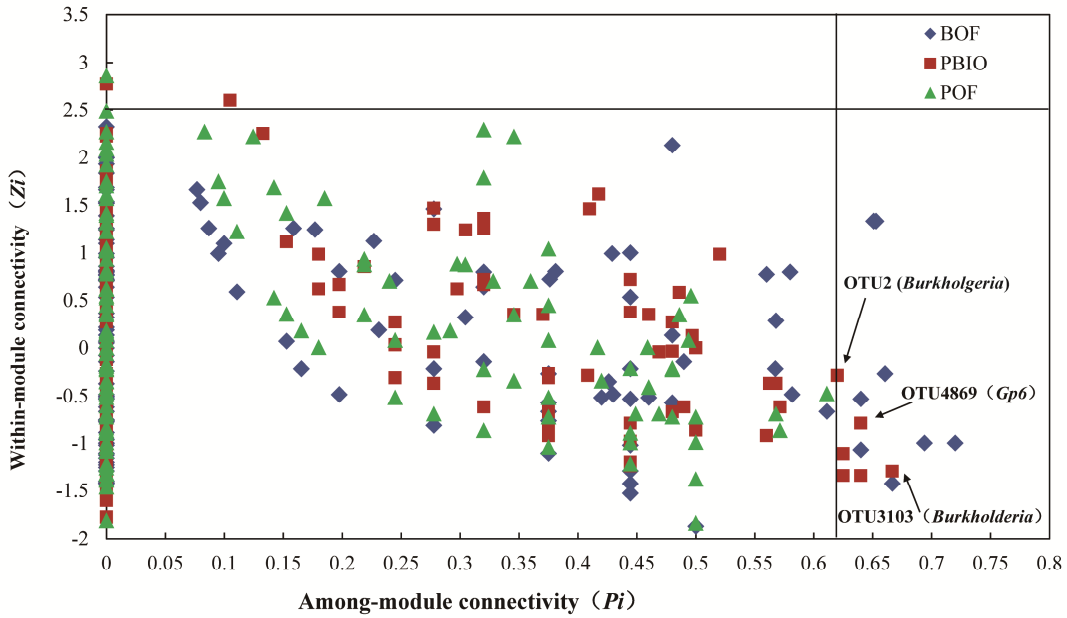

Figure 6. *Zi-Pi* plot showing the distribution of OTUs based on their topological roles. Each symbol represents an OTU in different treatment. BOF = banana continuously cropped with OF application; POF = banana-pineapple rotation with OF application in the banana season; PBIO = banana-pineapple rotation with BIO application in the banana season; The threshold values of *Zi* and *Pi* for categorizing OTUs were 2.5 and 0.62, respectively, as proposed by Guimera and Amaral (2005) and simplified by Olesen et al. (2007).

3.6 Relationship between microbial indicators and incidence of banana Fusarium wilt disease

Bacterial and fungal structure (unweighted PCoA1), richness (Chao1), and Faith's PD; Ascomycota, Gemmatimonadetes, and Nitrospirae phyla relative abundances; and *Fusarium*, *Burkholderia*, and *Bacillus* genus relative abundances were selected in the linear model and explored for the best contribution factor of disease incidence (**Table 3**).

Table 3 Linear models (LM) for the relationships of microbial indicators with disease incidence and the relative importance of each indicator. *p* was the result from ANOVAs. The bold values represent *p* values lower than 0.05 from the ANOVA results.

	df	*F*	*p*	Relative Importance
Bac-PCoA1	**1**	**304.09**	**<0.0001**	**19.32%**
Fun-PCoA1	1	1.11	0.31	16.32%
Bac-Chao1	1	4.10	0.062	9.60%
Fun-Chao1	1	1.11	0.309	4.19%
Bac-Faith's PD	1	1.59	0.227	6.80%
Fun-Faith's PD	1	1.64	0.221	6.05%
Ascomycota	1	2.11	0.168	1.93%
Fusarium Relative abundance	1	1.01	0.332	8.23%
Nitrospirae	1	0.88	0.363	7.29%
Gemmatimonadetes	1	0.04	0.852	2.61%
Burkholderia	1	0.76	0.399	10.17%
Bacillus	1	0.33	0.574	3.29%
Residuals	14			
Model summary: R^2=0.9417, AIC =123.26, $p < 0.0001$				
Proportion of variance explained by model: 95.79%				

Importantly, bacterial structure (F = 304.09, $p < 0.0001$, relative importance = 19.32%), fungal structure (F = 1.11, $p < 0.31$, relative importance = 16.32%), and *Burkholderia* relative abundance (F = 0.76, $p < 0.399$, relative importance = 10.17%) contributed most in constraining disease incidence (with a relative importance greater than 10%).

In addition, based on linear regression analyses between disease incidence and selected microbial indicators, we found that bacterial structure (F = 304.09, p < 0.0001, relative importance = 19.32%) had a significant relationship with disease incidence.

4 Discussion

In our previous research, the effectiveness of pineapple-banana rotation and biofertilizer application was proven in the control of banana Fusarium wilt disease (Wang et al., 2015; Fu et al., 2017). Soil microbial community change is an important indicator of exploring the mechanisms behind these two control measures. In this study, disease incidence and soil microbial community characteristics during the banana-growing season were measured to evaluate the control effect and potential impact of combined use of rotation and biofertilizer application.

Our previous results indicated that the pineapple-banana rotation treatments significantly reduced the Fusarium wilt disease incidence when compared with banana monoculture. Moreover, the application of biofertilizer enhances this suppression ability. Similar to our results, Shen et al. (2018) reported that bio-fertilizer application after fumigation with lime and ammonium bicarbonate revealed higher effectiveness in controlling banana Fusarium wilt disease compared to bio-fertilizer application or fumigation only. Although many control measures can slow down the spread of Fusarium wilt disease, more effective control can be achieved by the combined use of more than one measure (Pda et al., 2017). It was consistent with this paper.

In this study, Chao1 and Faith's PD were significantly higher in the combined rotation and biofertilizer treatment (PBIO) compared to the other two treatments (BOF and POF). Previous studies have shown a positive correlation between disease

409 suppression and bacterial but not fungal diversity (Bonanomi et al., 2010; Fu et al.,
410 2017). Inconsistent with these results, pineapple-banana rotation and biofertilizer
411 treatment (PBIO) harbored a significantly higher fungal richness and diversity than
412 the other two treatments (BOF and POF). This agrees with some other previous
413 studies that observed the importance of fungal diversity in the suppressive capacity of
414 vanilla soils and potato cropping systems (Lu et al., 2013; Xiong et al., 2017). We
415 also observed that soil pH was increased in the rotation and biofertilizer treatment
416 (**Table S2**). Many previous studies have shown that microbial diversity has been seen
417 to increase with higher soil pH values (Liu et al., 2014; Shen et al., 2013). Therefore,
418 the high bacterial and fungal diversity observed in PBIO treatment may be attributed
419 to the high soil pH.

420 Both PCoA ordinations and MRT results revealed significant differences in
421 microbial community structure after rotation and biofertilizer applications. This is
422 supported by previous studies stating that rotation (Helena et al., 2016; Hartmann et
423 al., 2015) and biofertilizer application (Sun et al., 2015) altered the soil microbial
424 community composition. MRT analysis also revealed fertilization effects on microbial
425 community composition. These results are similar to previous results where
426 biofertilizer application was the dominant factor in determining microbial community
427 composition rather than temporal variability (Fu et al., 2017), suggesting a powerful
428 illustration of the necessity of biofertilizer application in pineapple-banana rotation
429 system.

430 Phylum-level results show that rotation and biofertilizer application decreased
431 the relative abundance of Ascomycota, and increased the relative abundance of
432 Chlamydiae, Gemmatimondetes, Nitrospire, Planctomycetes, and Verrucomicrobia,
433 which were all reported to be associated with disease suppression in previous reports
434 (Trivedi et al., 2017; Shen et al., 2018).

435 It is worth noting that our BIO was secondary fermentation with *Bacillus* added,
436 while the *Bacillus* genus was not enriched in the BIO treatment soil. Moreover, the
437 microbial structure appeared to be the most constrained factor in disease incidence in
438 linear models between microbial indicators and the incidence of banana Fusarium wilt
439 disease. Xiong et al (2017) suggested that microbial species introduced by
440 biofertilizer application induced wilt suppression by microbiome manipulation rather
441 than pathogen suppression directly. Thus, alteration of the soil microbiome may cause
442 a greater response than the added *Bacillus* in the PBIO treatment in our case.
443 Compared with bacteria, a higher percentage of *Fusarium*-related fungi genera
444 were observed in all treatments. Even though more kinds of bacteria are related to
445 *Fusarium*, a higher percentage of fungi showed relevance compared to bacteria. These
446 results agree with the findings of Mona et al. (2014) and Cai et al. (2017), who
447 reported that fungal communities have a more crucial response to soil factor changes
448 than bacterial communities. It is worth noting that fungal communities were more
449 dissimilar between the pineapple-banana rotation and maize-banana rotation
450 treatments than bacteria in our previous study (Wang et al., 2015). Thus, the higher
451 *Fusarium*-relevance observed in the fungal community in both the pineapple and
452 banana seasons further reinforced the importance of fungal community changes in our
453 case.
454 Several researchers have used microbial molecular ecological networks to study
455 complex microbial ecological systems in suppressed soils, including corn-potato
456 rotations (Lu et al., 2013) and vanilla (Xiong et al., 2017). In our study, the microbial
457 molecular ecological networks revealed distinct differences between the microbial
458 communities associated with the three treatments. More fungal OTUs were selected in
459 the PBIO treatment samples, followed by the POF and BOF treatments, based on the
460 F/B ratio. Although the OTUs selected to build the network were only a part of the

entire system, there is no doubt that these OTUs were very important for soil function (Coyte et al., 2015). Therefore, we conclude that a large number of fungal OTUs present in the system may have led to changes in soil function. PBIO, POF, and BOF soils harbored modules with modularity values of 0.718, 0.642, and 0.616, respectively, in this study. Modularity represents how well the network was organized (Zhou et al., 2011). Thus, the PBIO network, which possessed high modularity, had more connections between nodes in the same modules, followed by the POF and BOF networks. The altered networks compared to POF and BOF networks may have partially contributed latent attributes to higher disease suppression in PBIO treatment. Furthermore, no module hubs (generalists) were present in the BOF network, whereas all three module hubs were found in the pineapple-banana rotation network, as indicated by the *Zi-Pi* relationship. In all three networks, connectors (generalists) and module hubs (generalists) were found at the same time only in the PBIO treatment. Generalists typically only occupy a small fraction of a community; however, the presence of those generalists is very important (Zhou et al., 2011; Jens et al., 2011). These nodes could have enhanced connectors within or among modules. If the network is poorly connected or not connected at all, the community is predicted to be disordered, and fluxes of energy, material, and information would not be efficient (Lu et al., 2013). Therefore, in our case, these generalists found in the PBIO treatment suggest that the microbial community structure of PBIO treatment was more orderly and powerful than the other two treatments.

Annotation information from all generalists found in our study shows that bacterial OTU2 and OTU3013 belong to *Burkholderia*, which were generalists in the PBIO but not in POF and BOF networks. The linear model analysis also shows that *Burkholderia* relative abundance constrained disease incidence with a higher relative importance factor of 10.17%. Correspondingly, a high abundance of *Burkholderia* and

a high percentage of antagonistic *Burkholderia* were found during the pineapple season in our previous report (Wang et al., 2015). *Burkholderia* is a versatile organism due to its powerful ability to occupy ecological niches and a variety of functions, including biological control and plant growth promotion in agriculture (Coenye and Vandamme, 2003). Thus, even though the relative abundance of *Burkholderia* in the PBIO treatment was not that high, its change in network structure may have been attributed to the general wilt suppression activity, which is the special function of *Burkholderia*. Additionally, one generalist in the PBIO treatment sample was identified as *Gp6* in Acidobacteria. Although no Acidobacteria antimicrobial activities have previously been recorded, several studies have demonstrated that Acidobacteria is greatly affected by soil pH and that the *Gp6* is positively correlated with soil pH (Bartram et al., 2014; Jones et al., 2009).

5 Conclusions

This study was an expansion of our previous work. The results revealed that pineapple-banana rotation combined with biofertilizer application during the banana season effectively reduced the *Fusarium* spp. abundance and banana Fusarium wilt. Both bacterial and fungal taxonomic and phylogenetic α-diversity was increased by rotation and biofertilizer application. Between the two strategies, biofertilizer application affected both bacterial and fungal community composition more predominantly compared to rotation. A higher percentage of *Fusarium*-related fungal phyla was observed compared to bacterial. More specifically, the potentially beneficial *Burkholderia* genus may attribute to the general wilt suppression activity for its important role in network structure and its high relative importance in linear models. Pineapple-banana rotation combined with biofertilizer application has strong potential for the sustainable management of banana Fusarium wilt disease.

Data availability

All data are available. The sequencing data have been submitted to the NCBI Sequence Read Archive database (SRP234066).

Author contributions

Rong Li and Beibei Wang designed the research and wrote the manuscript. Beibei Wang, Yannan Ou and Zongzhuan Shen performed trials and conducted field work. Beibei Wang and Jinming Yang analyzed the data. Rong Li, Mingze Sun, Lin Fu, Yunze Ruan, Yan Zhao and Qirong Shen participated in the design of the study, provided comments and edited the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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