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 bio-organic fertilizer application have historically been employed as efficient management strategies for soil-borne disease suppression through soil microbiome manipulation. However, details of how this occurs and to what extent the combination of methods affects soil microbiota reconstruction from diseased soils lack investigation. 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 that the application of bio-organic fertilizer caused additional suppression. Bacterial and fungal communities thrived using rotation in combination with bio-organic fertilizer application: taxonomic and phylogenetic α-diversity in both bacteria and fungi increased along with disease suppression. Between the two strategies, bio-organic fertilizer application affected both bacterial and fungal community composition most predominantly, followed by rotation. Large-scale changes in the fungal community composition and special *Burkholderia*-related network contributed to the observed soil-borne disease suppression. Our results indicated that pineapple-banana rotation combined with bio-organic fertilizer 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, which is 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 disease 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 focussed on using multiple strategies to improve control efficiency. Shen et al. (2018) reported that biofertilizer application after fumigation with lime and ammonium bicarbonate was an effective strategy in banana Fusarium wilt disease control. Thus, although 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.

~~Of all available~~ management strategies, chemical pesticides are optimally effective against soil-borne plant pathogens, but this strategy is not friendly to the environment, including polluting soil and water and inducing 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 a sustainable alternative to chemical pesticides (Alabouvette et al., 2009; Fravel et al., 2003; Qiu et al., 2012). Biofertilizers combine the advantage of introducing beneficial microbes that will occupy niches with the inclusion of organic material that will create additional niches for beneficial indigenous microbes (Cai et al., 2017; Zhang et al., 2013). In our previous study, we ~~investigated~~ a biofertilizer containing ~~a~~ *Bacillus* strain isolated from the rhizosphere of a continuously cropped banana, and we investigated its ability to promote banana growth and control Fusarium wilt in banana (Shen et al., 2015; Fu et al., 2016; Fu et al., 2017). Therefore, we concluded that bio-organic fertilizer application is a practicable and worthy measure for banana Fusarium wilt suppression.

In addition, crop rotation is also considered an alternative method in soil-borne disease control ~~because~~ it is highly efficient and environmentally friendly (Krupinsky et al., 2002). Crop rotation ~~often~~ breaks the microflora and chemical characteristics of ~~single~~ continuously cropped soil (Christen and Sieling, 2010; Yin et al., 2010). However, crop rotation has different effects on soil-borne diseases. The mechanisms of crop rotation control soil-borne disease include inhibiting the reproduction of pathogens through allelochemical secretion, inducing antagonistic microbes against pathogens, and improving rhizosphere microbial community structure by introducing different carbon compounds into the soil through root exudates or residues (Robert et al., 2014). In our previous work, banana-pineapple rotation was selected for its high-efficiency in banana Fusarium wilt disease prevention and control, as well as bio-organic fertilizer application (Wang et al., 2015). However, the combined control efficiencies of the two measures (pineapple-banana rotation and bio-organic fertilizer application) remain unknown. Thus, there is a great need to investigate efficient disease suppression-combined approaches for Fusarium wilt control in banana and hence to work 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 ~~FOC~~ minimization (Cha et al., 2016; Chaparro et al., 2012; Gerbore et al., 2014; Mazzola and Freilich, 2017). We proved the effectiveness of microbial agents for biocontrol by changing the structure of soil microbial communities in previous reports (Fu et al., 2017; Shen et al., 2015). We also investigated the influences of quarterly rotation (pineapple) on FOC population density and soil microbial community structure while attempting to explain 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 soil FOC decrease.

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 bio-organic fertilizer application) remains unknown.

We hypothesized that Fusarium wilt can be effectively controlled in high-incidence fields by pineapple-banana rotation and that the control efficiency can be increased when biocontrol is added to the rotation. In addition, this scheme will concurrently change 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 our rotation and biocontrol strategy.

2 Materials and Methods

2.1 Field experimental design

The field experimental site was set at Hainan Wanzhong Industrial Co., Ltd., China, a company that specialized in banana planting during December 2011and 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}. Fertilizer was supplied by Lianye Biofertilizer Engineering Center, Ltd., Jiangsu, China. The organic fertilizer (OF) used in our study was a first fermentation with a 2:3 weight ratio using amino acid fertilizer and pig manure. The bio-organic fertilizer (BIO) was a secondarily 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 a continuous banana cropping for more than 6 years. Nine replicates in each treatment were set up 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 applied (BOF); (2) banana planted after an eighteen-month pineapple rotation with common organic fertilizer applied in the banana season (POF); and (3) banana planted after an eighteen-month pineapple rotation treatment with bio-organic fertilizer applied (PBIO). In the rotation system, pineapple and banana were planted at densities of 45000 and 2,400 seedlings ha^{-1}, respectively. All organic fertilizer was applied to the soil at once as base fertilizer before banana planting; other measures were consistent with common banana production.

2.2 Banana Fusarium wilt disease incidence statistics

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

2.3 Soil sample collection and DNA extraction

During the harvest time of last banana season, 5 healthy plants were randomly picked out in each biological replicate plots for soil samples collection. Soil samples were collected from four random sites at a distance of 10 cm from banana plant, a depth of 20 cm soil column were picked out using soil borer in each sampling site. All the 5 soil columns from each biological replicate plots were mixed for DNA extraction. All mixed samples were placed in cold storage and transported to the laboratory. After screening used a 2-mm soil sieve, total soil DNA was extracted using Clean Soil DNA Isolation Kits (MoBio Laboratories Inc., Carlsbad, USA) from fine grained soil. After a determination of DNA concentration and quality using NanoDrop 2000 (Thermo Scientific, USA), all soil DNA were diluted to a concentration of 20 ng μl^{-1} for PCR amplification.

2.4 Polymerase chain reaction amplification and Illumina Miseq sequencing

Primers F520 (5’-AYTGGGYDTAAAGNG-3’) and R802 (5’-TACNVGGGTATCTAATCC-3’) were chosen to amplify the V4 regions of 16 S rRNA gene (Claesson et al., 2009). Primers ITS (5’-GGA AGT AAA AGT CGT AAC AAG G-3’) and ITS (5’-TCC TCC GCT TAT TGA TAT GC-3’) were chosen for amplification of the fungal ITS region (Schoch et al., 2012).

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

2.5 Bioinformatic analysis

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

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

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

Differences statistical analyses between the three treatments were carried out in SPSS 20.0 and R software. Pearson correlations among disease incidence, different Phylum and FOC relative abundance were analysed in R. Linear models were also proformed using R after stepwise model selection using Akaike information criteria.

3 Results

3.1 Disease incidence and relative abundance of *Fusarium*

Pineapple rotation and biofertilizer application effectively reduced Fusarium wilt disease incidence and the relative abundance of *Fusarium* in the next season's banana plantation (**Fig. 1A and B**). The incidences of banana Fusarium wilt disease in the

POF and PBIO treatments were 33.3% and 12.3%, respectively, which were significantly lower than those in the BOF treatment, which reached up to 66.8%. The treatment, PBIO with rotation and biofertilizer application, showed the lowest disease incidence with a 63.1% decrease compared with POF (**Fig. 1B and Table S1**). The relative abundance of *Fusarium* showed the same tendency as disease incidence, and disease incidence was significantly correlated with the relative abundance of *Fusarium*, as revealed by MiSeq sequencing data (**Fig. 1C**).

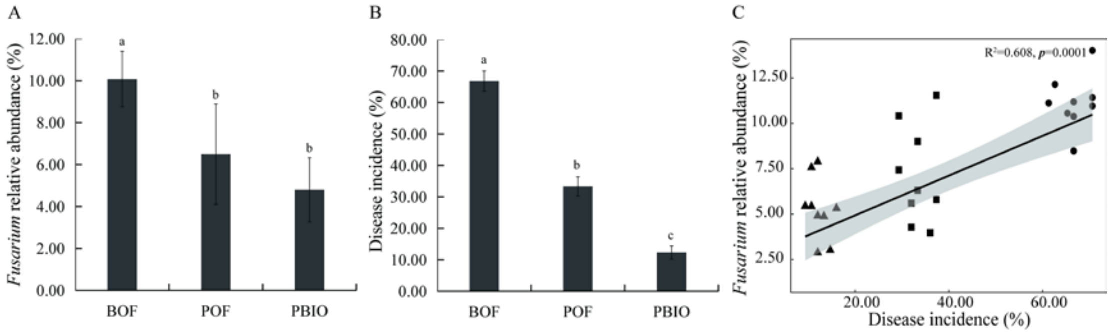

Figure 1. *Fusarium* relative abundance (A), Fusarium wilt disease incidence (B) and Pearson correlations between Fusarium wilt disease incidence and *Fusarium* relative abundance **(C).** BOF=banana continuously cropped with OF applied; POF= banana-pineapple rotation with OF applied in the banana season; and PBIO=banana-pineapple rotation with BIO applied in the banana season. Bars above the histogram represent standard errors and different letters indicate signifcant 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 relative abundances all greater than 1%. For fungi, Ascomycota, followed by Basidiomycota, Chytridiomycota, Zycomycota, and Glomeromycota were the most 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 in the BOF treatments, and the relative abundance of Ascomycota was lower in the PBIO treatment (Duncan test, *p* < 0.05).

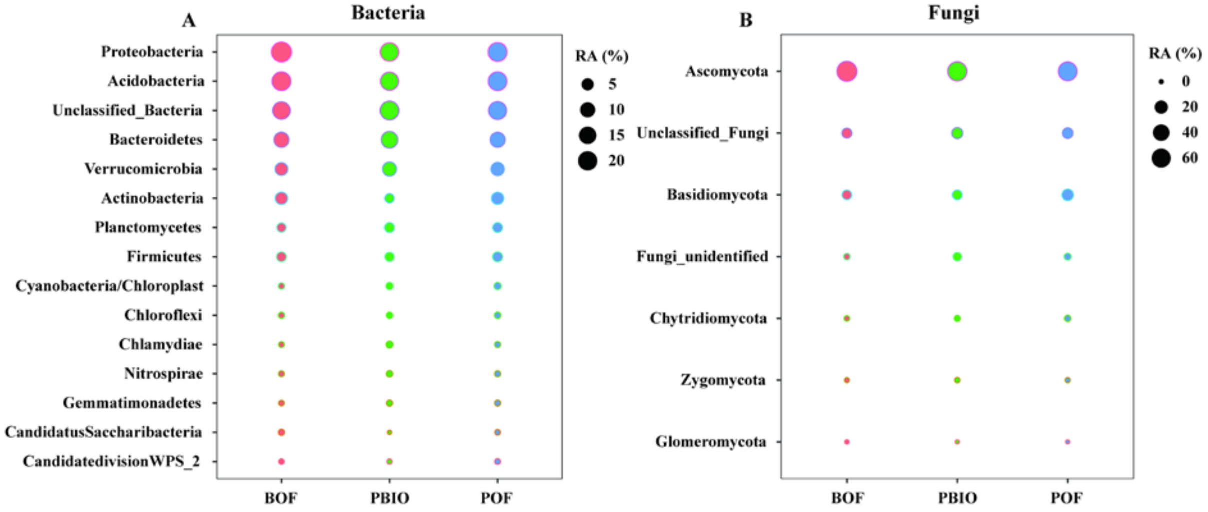

Figure 2. Bubble chart of bacterial (A) and fungal (B) phyla in BOF, POF and PBIO treatments. BOF=banana continuously cropped with OF applied; POF= banana-pineapple rotation with OF applied in the banana season; and PBIO=banana-pineapple rotation with BIO applied in the banana season; Values represent 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 with the

BOF treatment, more OTUs were observed in the POF and PBIO treatments, both for bacteria and fungi, and the PBIO treatment exhibited the most OTUs of all treatments (**Table 1**, **Fig. S1**). Compared with the BOF treatment, the pineapple-banana rotation treatments, POF and PBIO, increased both taxonomic and phylogenetic α-diversity in both bacteria and fungi. In addition, the 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 applied; POF= banana-pineapple rotation with OF applied in the banana season; PBIO=banana-pineapple rotation with BIO applied 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 in the three treatments. Fungal PCoA showed three distinct groups representing samples taken from the 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 that cultivation was secondarily important. Driven by fertilization, the PBIO treatment was separated from the BOF and POF treatments, and then the BOF and POF treatments were driven by cultivation (**Fig. 3B**).

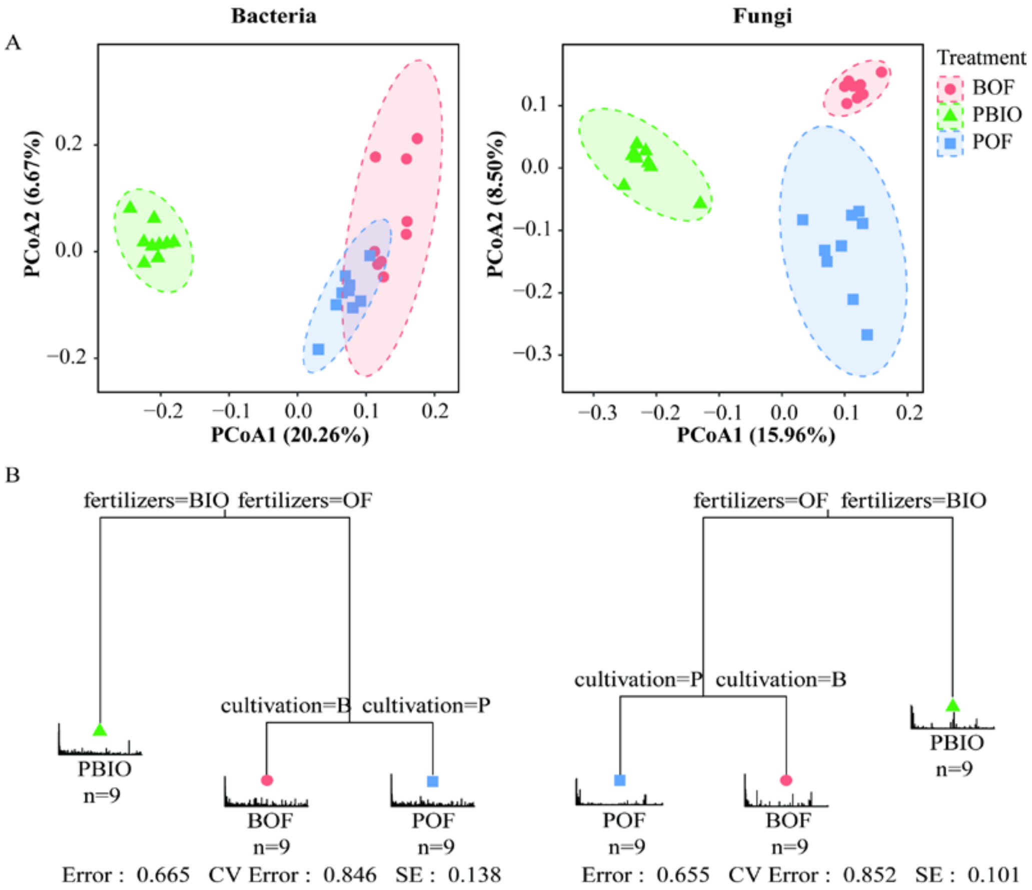

Figure 3. (A) UniFrac-unweighted principal coordinate analysis of fungal and bacterial community structures in different treatments. BOF=banana continuously cropped with OF applied; POF= banana-pineapple rotation with OF applied in the banana season; PBIO=banana-pineapple rotation with BIO applied in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

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

Phyla that were significantly correlated with FOC abundance were selected for the evaluation of effects on soil fungal and bacterial community composition versus relative FOC abundance. Seven bacterial phyla and three fungal phyla were significantly correlated with pathogen abundance (**Tables S3 and S4**). Moreover, more fungi were significantly correlated with FOC abundance compared with bacteria, based on the percentage of FOC related phyla showing this trend (**Fig. 4B**).

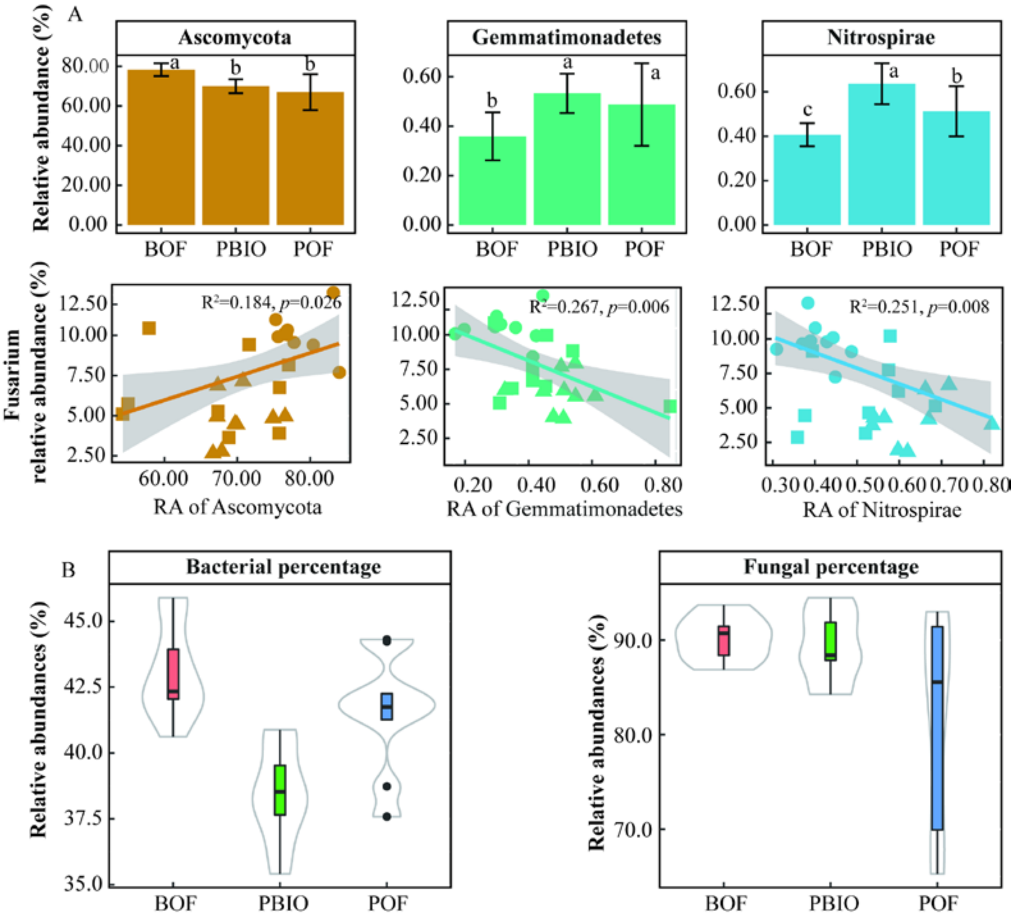

Figure 4. Relative abundance of key phyla and linear regression relationship between key phyla and disease incidence (A). Percentage of FOC related bacterial and fungal phyla in all treatments (B). Different letters above the bars indicate a significant difference at the 0.05 probability level according to the Duncan test.

3.5 Key topological properties of the networks

We built networks to show interactions among genera in the different treatments; those OTUs with a relative abundance greater than 0.1% were selected from each treatment. 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.

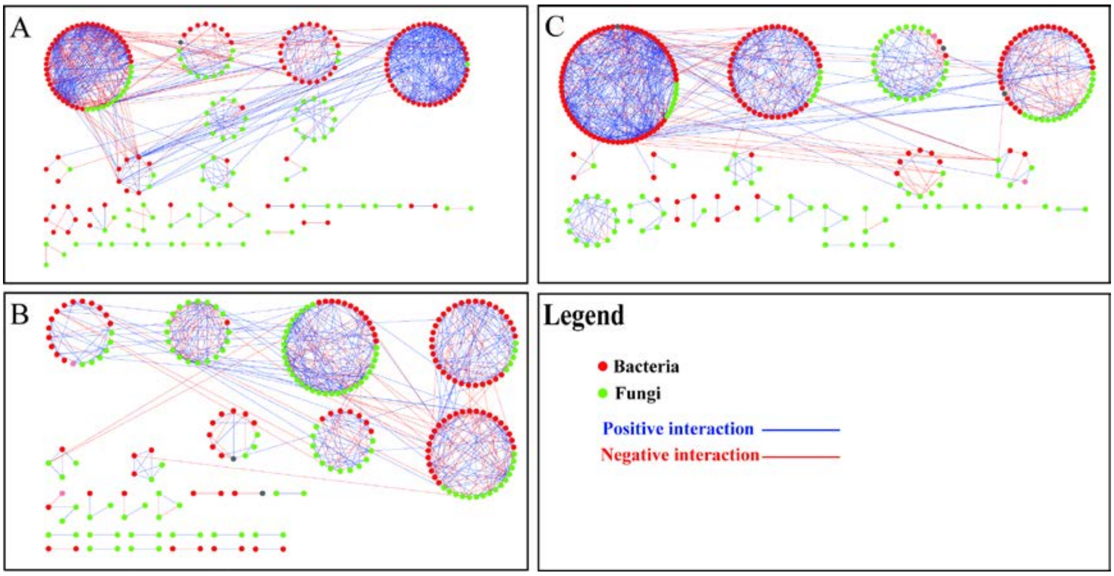

Figure 5. Network plots of bacterial and fungal communities in soil BOF (A), PBIO (B) and POF(C). BOF=banana continuously cropped with OF applied; POF= banana-pineapple rotation with OF applied in the banana season; PBIO=banana-pineapple rotation with BIO applied in the banana season; Red nodes indicate 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. F/B represents ratio of fungal to bacterial nodes. The F/B ratios 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 sample, followed by the POF and BOF treatments.

The structure index network from the 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 applied; POF= banana-pineapple rotation with OF applied in the banana season; PBIO=banana-pineapple rotation with BIO applied 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 were categorized as generalists (module hubs) with intense connectivity to many nodes in the same modules, two from the PBIO network and one from the POF network. However, no module hubs were 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 treatment (PBIO and POF), and connectors (generalists) and module hubs (generalists) were found at the same time only in the pineapple-banana with the bio-organic fertilizer 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.

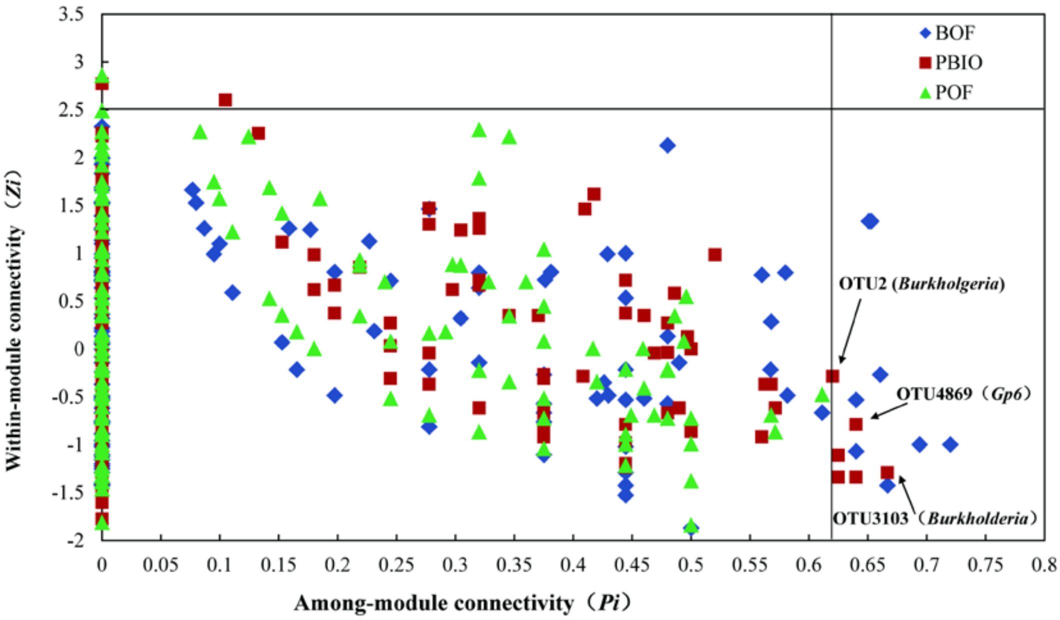

Figure 6. *Zi-Pi* plot showing the distribution of OTUs based on their topological roles. Each symbol represents an OTU in different treatment. BOF=banana continuously cropped with OF applied; POF= banana-pineapple rotation with OF applied in the banana season; PBIO=banana-pineapple rotation with BIO applied 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

Total response variance: 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%) constrained disease incidence the most (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 bio-organic fertilizer application were 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 for 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 rotation and bio-organic fertilizer application.

Our previous results indicated that the pineapple-banana rotation treatments significantly reduced Fusarium wilt disease incidence when compared with banana monoculture. Moreover, the application of bio-organic fertilizer enhances this suppression ability. Shen et al., (2018) reported that bio-fertilizer application after fumigation with lime and ammonium bicarbonate was highly effective in controlling banana Fusarium wilt disease. Thus, although many measures can slow down the spread of Fusarium wilt disease, effective control can be enhanced by the combined use of more than one measure (Pda et al., 2017). ~~Therefore, in the current study, we explored the combined use effect of pineapple-banana rotation and bio-organic fertilizer application to provide a promising strategy to manage banana Fusarium wilt disease. The results were consistent with previous reports.~~

Significantly higher Chao1 and Faith's PD were detected in the rotation and biofertilizer treatments. Previous studies have shown a high positive correlation between disease suppression and a high diversity of bacteria with a concurrent low diversity of fungi (Bonanomi et al., 2010; Fu et al., 2017). However, inconsistent with these results, the pineapple-banana rotation and biofertilizer treatment (PBIO) harbored a significantly higher fungal richness and diversity than the other two treatments (BOF and POF). This agrees with two other previous studies that indicated the importance of fungal diversity in the suppressive capacity of vanilla soils and potato cropping systems (Xiong et al., 2017). Many previous studies have shown that a decrease in soil pH is an important factor leading to soil-borne diseases. Microbial diversity has been seen to increase with higher soil pH values (Liu et al., 2014; Shen et al., 2013). We observed that soil pH increased in the rotation and bio-organic fertilizer treatments (**Table S2**); therefore, the high bacterial and fungal diversity observed in our rotation and bio-organic fertilizer system may have been due to the high soil pH.

Both PCoA ordinations and MRT results revealed significant differences in microbial community structure after rotation and biofertilizer applications. This is supported by previous studies stating that rotation (Helena et al., 2016; Hartmann et al., 2015) and bio-organic fertilizer application (Sun et al., 2015) altered the soil microbial community composition. Despite the apparent cultivation, MRT analysis revealed fertilization effects on microbial community composition, indicating that bio-organic fertilizer application in the banana season was the most important factor in determining microbial community composition. These results were similar to previous results where bio-organic fertilizer application was the greatest factor in determining microbial community composition rather than temporal variability (Fu et al., 2017). This is also a powerful illustration of the necessity of bio-organic fertilizer application in pineapple-banana rotation.

Phylum-level results show that rotation and biofertilizer application decreased the relative abundance of Ascomycota, and increased the relative abundance of Chlamydiae, Gemmatimondetes, Nitrospire, Planctomycetes, and Verrucomicrobia, which were all associated with disease suppression in previous reports (Trivedi et al., 2017; Shen et al., 2018). Our fungal results are consistent with previous observations of low Ascomycota phylum abundance in suppressed soil, which is logical because Ascomycetes constitutes the largest group of soil pathogens (Lu et al., 2013). Furthermore, this tendency was observed in our previous report, in which a decrease in Ascomycetes was considered an important factor in FOC decrease during the pineapple season (Wang et al., 2015). The bacterial results are partly consistent with previous observations that rotations with wild rocket and Indian mustard increased the *Nitrospira* and Gemmatimonadetes contents (Jin et al., 2019). It is worth noting that our BIO treatment was secondary fermentation with *Bacillus* added, while the *Bacillus* genus was not enriched in the BIO treatment soil. Moreover, microbial structure appeared to be the most constrained factor with disease incidence in linear models between microbial indicators and the incidence of banana Fusarium wilt disease. Xiong et al (2017) suggested that microbial species introduced by biofertilizer application induced wilt suppression by microbiome transformation rather than pathogen suppression directly. Alteration of the soil microbiome may cause a greater response than the added *Bacillus* in the PBIO treatment sample in our case.

We previously confirmed that pineapple-banana rotation reduced the amount of *Fusarium oxysporum* mainly by modulating fungal communities during the pineapple season (Wang et al., 2015). In the present research, compared with bacteria, a higher percentage of FOC-correlated fungi genera was observed in all treatments. Even though more kinds of bacteria are related to FOC, a higher percentage of fungi showed relevance. These results agree with the findings of Mona et al. (2014) and Cai et al. (2017), who reported that fungal communities have a more crucial response to soil factor changes than bacterial communities. It is worth noting that fungal communities were more dissimilar between the pineapple-banana rotation and maize-banana rotation treatments than bacteria in our previous studies (Wang et al., 2015). Thus, the higher FOC-relevance found in the fungal community in both the pineapple and banana seasons further reinforced the importance of fungal community changes in our case.

Several researchers have used microbial molecular ecological networks to study complex microbial ecological systems in suppressed soils, including corn-potato rotations (Lu et al., 2013) and vanilla (Xiong et al., 2017). We found microbial molecular ecological networks revealing distinct differences between the microbial communities associated with the three treatments in our research. More fungal OTUs were selected in the PBIO treatment samples, followed by the POF and BOF treatments, based on the 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 the 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 with POF and BOF networks may have partially contributed latent attributes to higher disease suppression in our rotation and bio-organic fertilizer application trials. 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 connecters 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 was more orderly and powerful than in 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 network, but were not observed in the POF and BOF networks. 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). In addition, our linear model analysis shows that in addition to bacterial and fungal structure, *Burkholderia* relative abundance constrained disease incidence with a high relative importance factor of 10.17%. *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). This suggests that even though the relative abundance of *Burkholderia* in the PBIO treatment was not that high, the change in network structure in the rotation and bio-organic fertilizer treatments may have been attributed to the general wilt suppression activity, and that change may have specifically been due to the special functions 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 *Gp6* is positively correlated with soil pH (Bartram et al., 2014; Jones et al., 2009). Therefore, the special function of *Gp6* in the PBIO network probably resulted from an increase in soil pH.

5 Conclusions

An expansion of previous work revealed that pineapple-banana rotation combined with bio-organic fertilizer application during the banana season was effective in reducing *Fusarium* spp. abundance and banana Fusarium wilt. Several different analyses indicate that bacterial and fungal communities, especially fungal structure, were changed by rotation and bio-organic fertilizer application. Bio-organic fertilizer inhibited Fusarium wilt disease by changing the soil microbial structure, rather than any designated microorganism. Large changes in the fungal community and special *Burkholderia* functions in the network were likely the most responsible factors for soil-borne disease suppression. Pineapple-banana rotation combined with bio-organic fertilizer 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, YannanOu and ZongzhuanShen performed trials and conducted fieldwork. Beibei Wang and Jinming Yang analyzed the data. Rong Li, 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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