1 **Inducing Banana Fusarium Wilt Disease Suppression through Soil**

2 **Microbiome Reshaping by Pineapple-Banana Rotation Combined**

3 **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 Illumina 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;

75 Zhang et al., 2013). In our previous study, we developed a biofertilizer containing the

76 *Bacillus* strain isolated from the rhizosphere of a continuously cropped banana that

77 promoted plant growth and suppressed the banana Fusarium wilt (Shen et al., 2015;

78 Fu et al., 2016; Fu et al., 2017). Therefore, biofertilizer application is a practicable

79 and worthy measure for banana Fusarium wilt suppression.

80   In addition, crop rotation is also considered a highly efficient and

81 environmentally friendly alternative method in soil-borne disease control (Krupinsky

82 et al., 2002). Crop rotation breaks the microflora and chemical characteristics of

83 continuously mono-cropped soil leading to the control of soil-borne diseases (Christen

84 and Sieling, 2010; Yin et al., 2010). The mechanisms of soil-borne disease

85 suppression induced by crop rotation include inhibition of pathogen reproduction

86 through allelochemical secretion, stimulation of antagonistic microbes against

87 pathogens, and improvement of rhizosphere microbial community structure by

88 introducing different carbon compounds into the soil through root exudates or

89 residues (Robert et al., 2014). In our previous study, besides biofertilizer application,

90 our work also showed that the banana-pineapple rotation efficiently suppressed the

91 banana Fusarium wilt disease (Wang et al., 2015). However, the combined control

92 efficiency of the two measures (pineapple-banana rotation plus biofertilizer

93 application) remains unknown. Thus, there is a great need to explore efficient disease

94 suppressing-combined approaches for the banana Fusarium wilt control and to

95 progress towards maintaining sustainable worldwide industrial banana development.

96   The occurrence of soil-borne disease is mainly due to the imbalance of soil

97 microbial communities caused by soil-borne pathogen blooms (Mendes et al., 2014).

98 Effective soil-borne disease suppression management strategies must demonstrate

99 significant changes in the soil microbial community in addition to pathogen

100 minimization (Cha et al., 2016; Chaparro et al., 2012; Gerbore et al., 2014; Mazzola

101 and Freilich, 2017). Our previous reports proved the effectiveness of microbial agents

102 for biocontrol by changing the structure of soil microbial communities (Fu et al., 2017;

103 Shen et al., 2015). We also investigated the influences of quarterly rotation (pineapple)

104 on *Fusarium* population density and soil microbial community structure while

105 attempting to explore the mechanisms of pineapple-banana rotation on soil borne-

106 disease suppression (Wang et al., 2015). Our results suggested that fungal community

107 structure and several genera introduced in the rotation season may have been the most

108 critical factors in decreasing soil *Fusarium* population.

109   Unlike intercropping, controlling *Fusarium* pathogen accumulation through

110 effective crop rotation should be maintained for at least two seasons, including

111 rotation and a subsequent season (Bullock, 1992; Lupwayi et al., 1998). The

112 pineapple and banana growth cycles in our rotation pattern require long durations

113 (almost 15 and 10 months, respectively, in Hainan Province, China). Thus, the soil

114 microbial community structure of the original season is very important in evaluating

115 rotation validity. Furthermore, how the soil microbial community structure changes

116 using the combined control efficiencies of the two measures (pineapple-banana

117 rotation and biofertilizer application) remains unknown.

118   We hypothesized that Fusarium wilt can be effectively controlled in high-

119 disease incidence fields by pineapple-banana rotation and that the control efficiency

120 can be improved by adding biocontrol to the rotation. In addition, this scheme will

121 concurrently change the soil microbial community membership and structure.

122 Therefore, based on our previous research, we conducted field experiments to

123 investigate the effects of pineapple-banana rotation combined with biofertilizer on

124 next season banana Fusarium wilt disease suppression and soil microbial communities.

125 Our objectives were to 1) determine the direct effects of pineapple-banana rotation

126 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

226 application showed the lowest disease incidence with a 63.1% decrease compared to

227 POF (**Fig. 1B and Table S1**). The relative abundance of *Fusarium* showed the same

228 tendency with disease incidence, so the relative abundance of *Fusarium* and disease

229 incidence were significantly correlated as revealed by MiSeq sequencing data (**Fig.**

230 **1C**).

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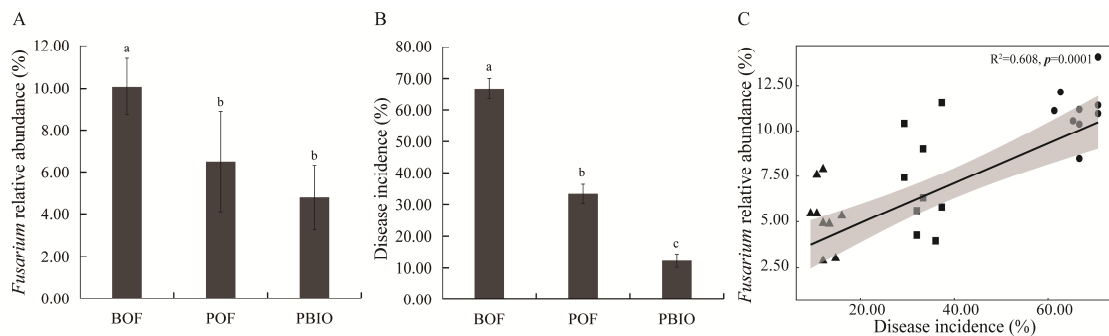
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233 **Figure 1.Relative abundance of *Fusarium* (A), Fusarium wilt disease incidence (B) and**

234 **Pearson correlations between Fusarium wilt disease incidence and *Fusarium* relative**

235 **abundance (C).** BOF = banana continuously cropped with OF application; POF = banana-

236 pineapple rotation with OF application in the banana season; and PBIO = banana-pineapple

237 rotation with BIO application in the banana season. Bars above the histogram represent

238 standard errors and different letters indicate significant differences ($p < 0.05$) according to

239 multivariate variance analysis and multiple comparison results.

240 **3.2 General analyses of the high-throughput sequencing data**

241 After quality control, 908,506 *16S rRNA* and 1,950,262 ITS sequences were retained

242 and based on 97% similarity, a total of 8,346 *16S* and 5,647 ITS operational

243 taxonomic units (OTUs) were obtained. For bacteria, Acidobacteria, Actinobacteria,

244 Bacteroidetes, Firmicutes, Proteobacteria and Verrucomicrobia were the most

245 abundant phyla with >1% relative abundances. For fungi, Ascomycota, followed by

246 Basidiomycota, Chytridiomycota, Zycomycota, and Glomeromycota were the

247 abundant phyla (**Fig. 2**). ANOVA showed that Chlamydiae, Cyanbacteria/chloroplast,

248   Gemmatimonadetas, Nitrospirae, Planctmycetes, and Verrucomicrobia abundances

249   were significantly higher in the PBIO and POF treatment samples than those in the

250   BOF treatment, and the relative abundance of Ascomycota was lower in the PBIO

251   treatment (Duncan test, $p < 0.05$).

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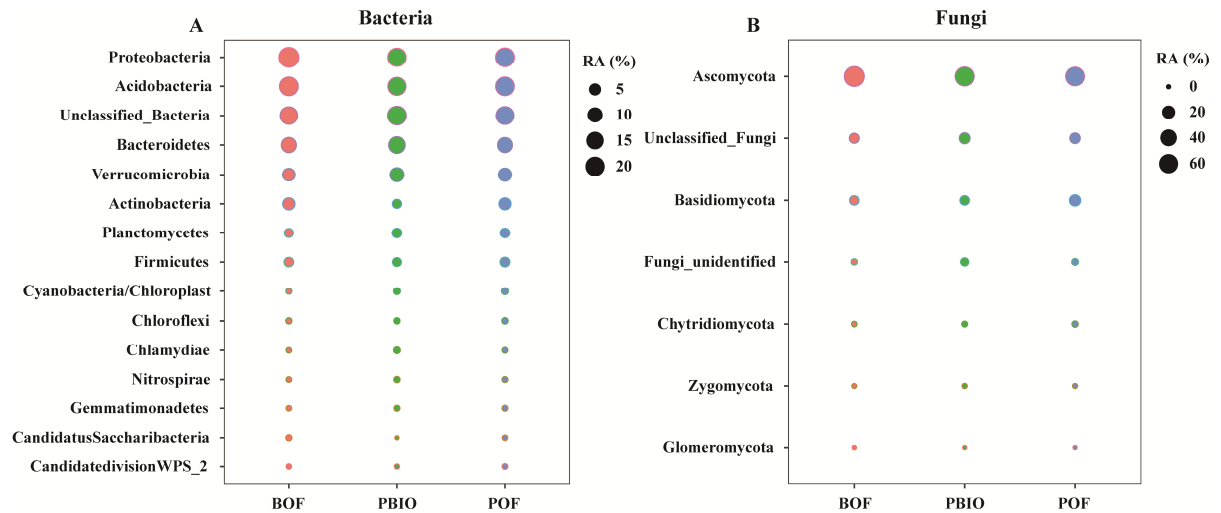
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254   **Figure 2. Bubble chart of bacterial (A) and fungal (B) phyla in BOF, POF and PBIO**

255   **treatments.** BOF = banana continuously cropped with OF application; POF = banana-

256   pineapple rotation with OF application in the banana season; and PBIO = banana-pineapple

257   rotation with BIO application in the banana season; Values represent the average abundance

258   across the nine replicate libraries for soil samples collected from each treatment.

259

260   **3.3 Effect of pineapple rotation and biofertilizer application on soil microbial**

261   **diversity and community structure**

262   Rarefaction analyses, Chao1 and Faith's PD were performed to characterize α-

263   diversity. Rarefaction analyses showed that the number of OTUs tended to smooth at

264   14,900 selected bacterial sequences and 34,943 fungal sequences. Compared to BOF

265   treatment, more OTUs were observed in POF and PBIO treatments, both for bacteria

266   and fungi, and the PBIO treatment exhibited the highest value of all treatments (**Table**

267   **1**, **Fig. S1**). Compared to BOF treatment, the pineapple-banana rotation treatments,

268   POF and PBIO, increased both taxonomic and phylogenetic α-diversity of both

269   bacteria and fungi. In addition, PBIO treatment showed the highest Chao1 richness

270   and Faith's PD values (**Table 1**).

271   **Table 1.** Bacterial and fungal α-diversity indexes of the three treatments. BOF = banana

272   continuously cropped with OF application; POF = banana-pineapple rotation with OF

273   application in the banana season; PBIO = banana-pineapple rotation with BIO application in

274   the banana season; Values represent the average index of nine replicates. Means followed by

275   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

276

277   We evaluated microbial community structure by using PCoA based on a UniFrac

278   unweighted distance matrix to analyze differences in community composition of three

279   treatments. Fungal PCoA showed three distinct groups representing samples taken

280   from three treatments; however, bacterial PCoA showed only two groups.

281   Unweighted UniFrac distances showed that PBIO treatment was separate from BOF

282   and POF treatments along the first component (PCoA1) both in bacteria and fungi.

283   POF treatment was separated from BOF treatment along the second component in

284   fungi, whereas in bacteria, POF and BOF treatments were not separate along the

285   second component (**Fig. 3A**).

286   Furthermore, MRT results indicated that biofertilizer application had the largest

287   deterministic influence on the composition of both bacterial and fungal communities,

288   and cultivation was secondarily important. Driven by fertilization, PBIO treatment

289   was separated from BOF and POF treatments, and then BOF and POF treatments

290   were driven by cultivation (**Fig. 3B**).

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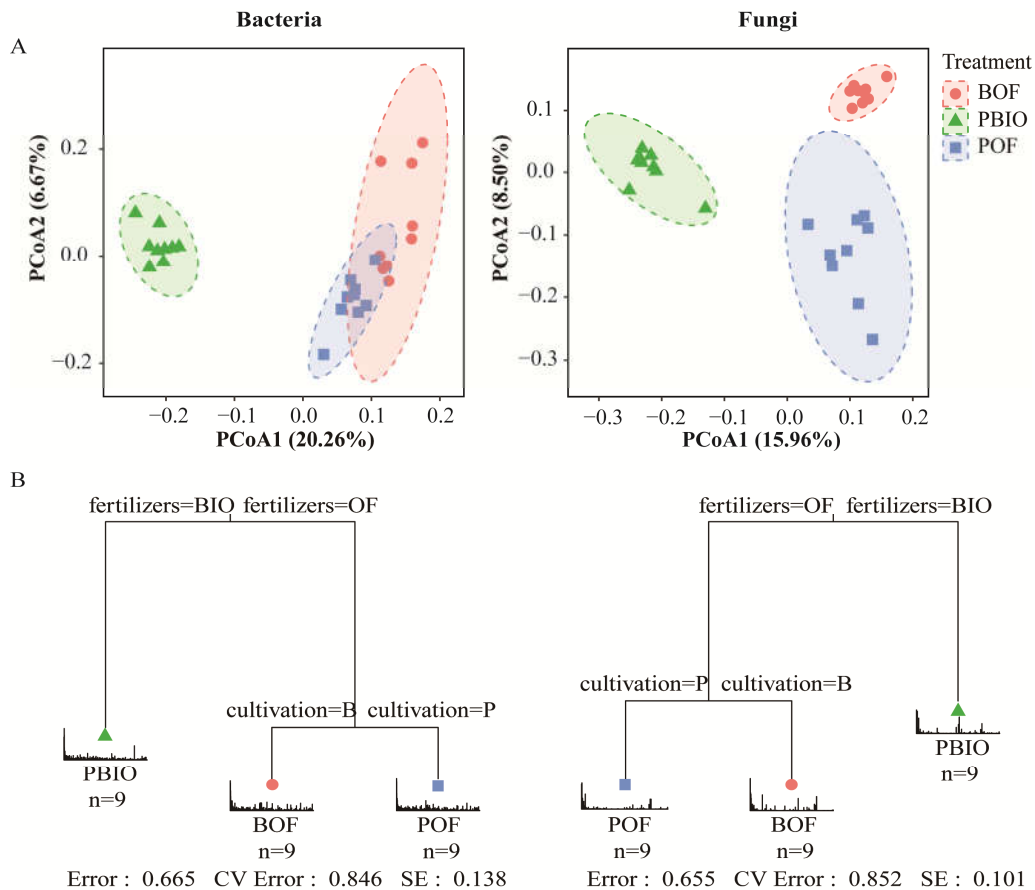

292   **Figure 3. (A) UniFrac-unweighted principal coordinate analysis of fungal and bacterial**

293   **community structures in different treatments. (B) Multiple regression tree (MRT)**

294   **analysis for the bacterial and fungal communities showed the variables of fertilization**

295   **and cultivation in each branch.** BOF = banana continuously cropped with OF application;

296   POF = banana-pineapple rotation with OF application in the banana season; PBIO = banana-

297   pineapple rotation with BIO application in the banana season.

298   **3.4 Effect of pineapple rotation and biofertilizer application on soil fungal and**

299   **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**).

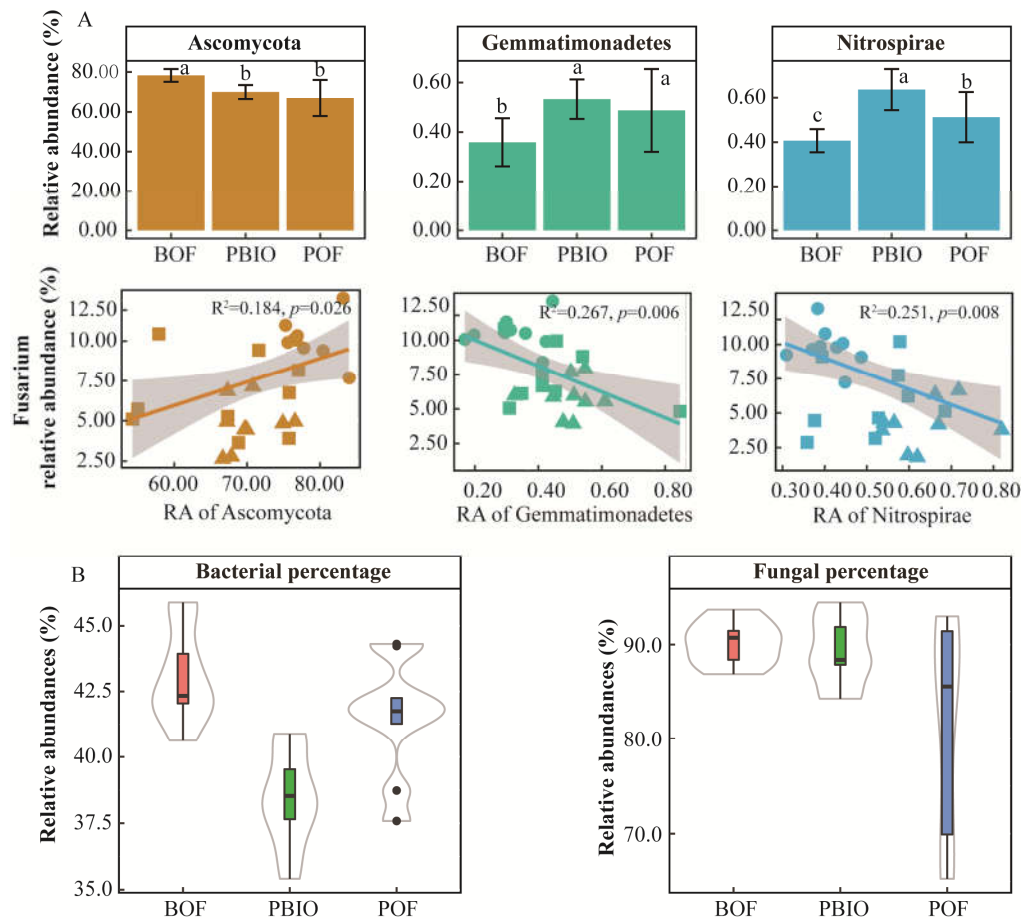



Figure 4. Relative abundance of key phyla and linear regression relationship between key phyla and disease incidence (A). Percentage of *Fusarium* related bacterial and fungal phyla in all treatments (B). BOF = banana continuously cropped with OF application; POF = banana-pineapple rotation with OF application 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

313 We built networks to show interactions among genera in different treatments using

314 OTUs with a relative abundance greater than 0.1%. A total of 301 OTUs were

315 selected from the BOF treatment (122 bacterial and 179 fungal), 323 OTUs were

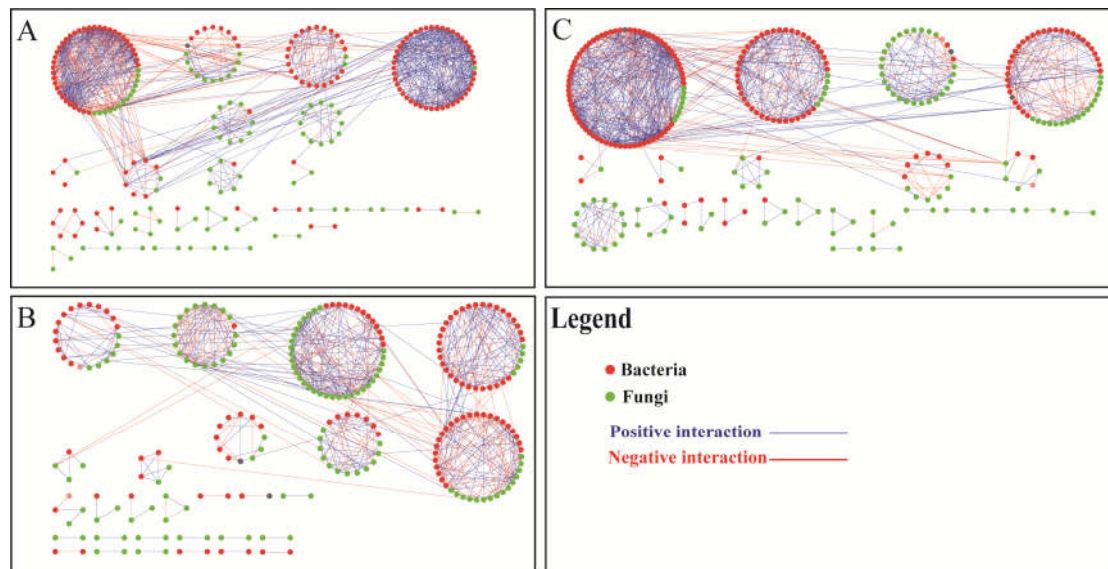
316 selected from the PBIO treatment (152 bacterial and 171 fungal), and 324 OTUs were

317 selected from the POF treatment (140 bacterial and 184 fungal). Random matrix

318 theory was used to build the networks. As shown in **Fig. 5**, each node represents an

319 OTU, each link shows a significant correlation between two OTUs, red and green

320 represent bacterial and fungal OTUs, respectively, and blue and red represent positive

321 and negative correlations, respectively.

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323 **Figure 5. Network plots of bacterial and fungal communities in soil BOF (A), PBIO (B)**

324 **and POF(C).** BOF = banana continuously cropped with OF application; POF = banana-

325 pineapple rotation with OF application in the banana season; PBIO = banana-pineapple

326 rotation with BIO application in the banana season; Red nodes indicate bacteria; Green nodes

327 indicate fungi; red lines between nodes (links) indicate negative interaction; and blue lines

328 indicate positive interaction.

329

330   Networks with 286 (143 bacterial and 98 fungal), 245 (122 bacterial and 123

331 fungal), and 241 (163 bacterial and 123 fungal) nodes were selected from the BOF,

332 PBIO, and POF treatments, respectively. The F/B ratios that represent the ratio of

333 fungal to bacterial nodes were 0.69, 1.01, and 0.75 in the BOF, PBIO, and POF

334 treatments, respectively. These results suggest more active fungal OTUs in the PBIO

335 treatment followed by the POF and BOF treatments.

336   The structure index network from different treatments showed 24, 28, and 30

337 modules in the BOF, PBIO, and POF treatments, respectively (**Table 2**).

338 **Table 2.** Topological properties of the empirical and associated random pMENs of microbial

339 communities under BOF, POF and PBIO. BOF = banana continuously cropped with OF

340 application; POF = banana-pineapple rotation with OF application in the banana season;

341 PBIO = banana-pineapple rotation with BIO application in the banana season; Avg K =

342 average connectivity; Avg CC = average clustering coefficient; and GD = average path

343 distance.

Treatment	Network size	R^2	Empirical networks				Random networks		
			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

344

345   The threshold value *Zi* measures the connected degree between two nodes in the

346 same module, and *Pi* measures the connected degree between two nodes from

347 different modules. According to the *Zi* and *Pi* values found in our study, all nodes

348 were divided into four categories (**Fig. 6**). Three nodes, two from the PBIO network

349 and one from the POF network, were categorized as generalists (module hubs) with

350 intense connectivity to many nodes in the same modules. However, there was no

351 module hub found in the BOF network. Fourteen nodes were categorized as

352 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.
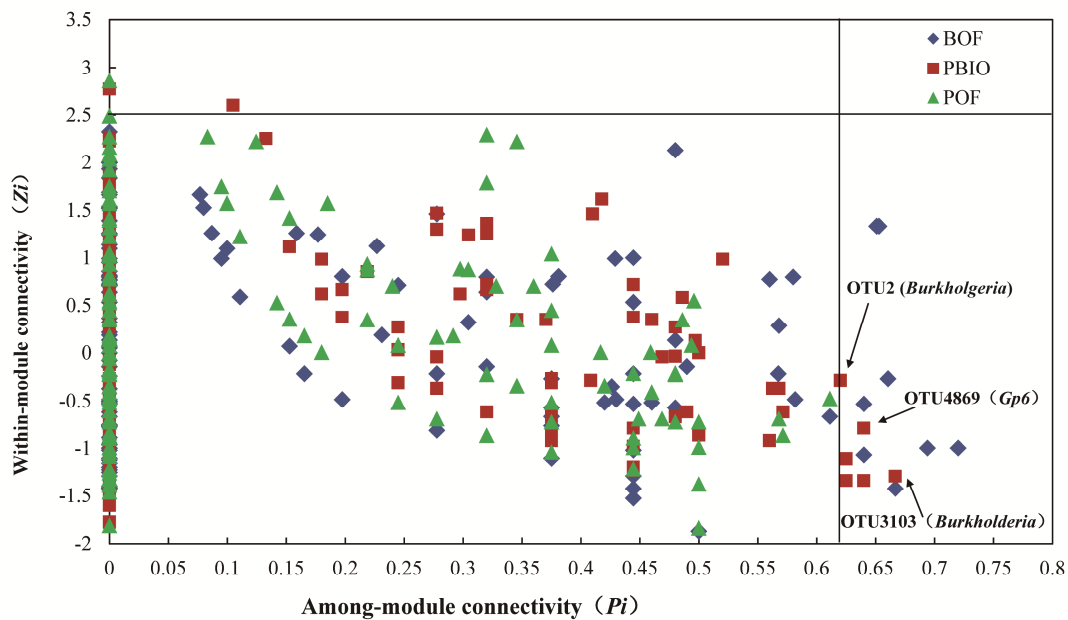
Figure 6. *Zi-Pi* plot showing the distribution of OTUs based on their topological roles. Each symbol represents an OTU in different treatment. BOF = banana continuously cropped with OF application; POF = banana-pineapple rotation with OF application in the banana season; PBIO = banana-pineapple rotation 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

370   Bacterial and fungal structure (unweighted PCoA1), richness (Chao1), and Faith's PD;

371   Ascomycota, Gemmatimonadetes, and Nitrospirae phyla relative abundances; and

372   *Fusarium*, *Burkholderia*, and *Bacillus* genus relative abundances were selected in the

373   linear model and explored for the best contribution factor of disease incidence (**Table**

374   **3**).

375   **Table 3** Linear models (LM) for the relationships of microbial indicators with disease
376   incidence and the relative importance of each indicator. p was the result from ANOVAs. The
377   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%				

378

379   Importantly, bacterial structure (F = 304.09, p < 0.0001, relative importance =

380   19.32%), fungal structure (F = 1.11, p < 0.31, relative importance = 16.32%), and

381   *Burkholderia* relative abundance (F = 0.76, p < 0.399, relative importance = 10.17%)

382   contributed most in constraining disease incidence (with a relative importance greater

383   than 10%).

384   In addition, based on linear regression analyses between disease incidence and
385   selected microbial indicators, we found that bacterial structure ($F = 304.09$, $p <$
386   0.0001, relative importance = 19.32%) had a significant relationship with disease
387   incidence.

388   **4 Discussion**

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

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

406   In this study, Chao1 and Faith's PD were significantly higher in the combined
407   rotation and biofertilizer treatment (PBIO) compared to the other two treatments
408   (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

461 entire system, there is no doubt that these OTUs were very important for soil function

462 (Coyte et al., 2015). Therefore, we conclude that a large number of fungal OTUs

463 present in the system may have led to changes in soil function. PBIO, POF, and BOF

464 soils harbored modules with modularity values of 0.718, 0.642, and 0.616,

465 respectively, in this study. Modularity represents how well the network was organized

466 (Zhou et al., 2011). Thus, the PBIO network, which possessed high modularity, had

467 more connections between nodes in the same modules, followed by the POF and BOF

468 networks. The altered networks compared to POF and BOF networks may have

469 partially contributed latent attributes to higher disease suppression in PBIO treatment.

470 Furthermore, no module hubs (generalists) were present in the BOF network, whereas

471 all three module hubs were found in the pineapple-banana rotation network, as

472 indicated by the *Zi-Pi* relationship. In all three networks, connectors (generalists) and

473 module hubs (generalists) were found at the same time only in the PBIO treatment.

474 Generalists typically only occupy a small fraction of a community; however, the

475 presence of those generalists is very important (Zhou et al., 2011; Jens et al., 2011).

476 These nodes could have enhanced connectors within or among modules. If the

477 network is poorly connected or not connected at all, the community is predicted to be

478 disordered, and fluxes of energy, material, and information would not be efficient (Lu

479 et al., 2013). Therefore, in our case, these generalists found in the PBIO treatment

480 suggest that the microbial community structure of PBIO treatment was more orderly

481 and powerful than the other two treatments.

482   Annotation information from all generalists found in our study shows that

483 bacterial OTU2 and OTU3013 belong to *Burkholderia*, which were generalists in the

484 PBIO but not in POF and BOF networks. The linear model analysis also shows that

485 *Burkholderia* relative abundance constrained disease incidence with a higher relative

486 importance factor of 10.17%. Correspondingly, a high abundance of *Burkholderia* and

487 a high percentage of antagonistic *Burkholderia* were found during the pineapple

488 season in our previous report (Wang et al., 2015). *Burkholderia* is a versatile

489 organism due to its powerful ability to occupy ecological niches and a variety of

490 functions, including biological control and plant growth promotion in agriculture

491 (Coenye and Vandamme, 2003). Thus, even though the relative abundance of

492 *Burkholderia* in the PBIO treatment was not that high, its change in network structure

493 may have been attributed to the general wilt suppression activity, which is the special

494 function of *Burkholderia*. Additionally, one generalist in the PBIO treatment sample

495 was identified as *Gp6* in Acidobacteria. Although no Acidobacteria antimicrobial

496 activities have previously been recorded, several studies have demonstrated that

497 Acidobacteria is greatly affected by soil pH and that the *Gp6* is positively correlated

498 with soil pH (Bartram et al., 2014; Jones et al., 2009).

499 **5 Conclusions**

500 This study was an expansion of our previous work. The results revealed that

501 pineapple-banana rotation combined with biofertilizer application during the banana

502 season effectively reduced the *Fusarium* spp. abundance and banana Fusarium wilt.

503 Both bacterial and fungal taxonomic and phylogenetic α-diversity was increased by

504 rotation and biofertilizer application. Between the two strategies, biofertilizer

505 application affected both bacterial and fungal community composition more

506 predominantly compared to rotation. A higher percentage of *Fusarium*-related fungal

507 phyla was observed compared to bacterial. More specifically, the potentially

508 beneficial *Burkholderia* genus may attribute to the general wilt suppression activity

509 for its important role in network structure and its high relative importance in linear

510 models. Pineapple-banana rotation combined with biofertilizer application has strong

511 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.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (41867006 and 31760605), the Hainan Provincial Natural Science Foundation of China (320RC483), the National Key Research and Development Program of China (2017YFD0202101).

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