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 lacks investigation. In this study, pineapple-banana rotation combined with biofertilizer application was used to suppress banana Fusarium wilt disease, and effects on both bacterial and fungal communities were investigated using the Miseq Illumine sequencing platform. Our results show that pineapple-banana rotation significantly reduces Fusarium wilt disease incidence, and that the application of bio-organic fertilizer causes additional suppression. Bacterial and fungal communities thrive using rotation in combination with bio-organic fertilizer application: taxonomic and phylogenetic α-diversity in both bacteria and fungi increase along with disease suppression. Between the two strategies, bio-organic fertilizer application affects both bacterial and fungal community composition most predominantly, followed by rotation. Large-scale changes in the fungal community composition and special *Burkholderia*-related network functions contribute to the observed soil borne-disease suppression. Our results indicate that pineapple-banana rotation combined with bio-organic fertilizer application has strong potential for the sustainable management of banana Fusarium wilt disease.

1 Introduction

Banana Fusarium wilt disease, which caused by *Fusarium oxysporum* f. sp. *cubense* (FOC) race 4 forms a major constraint for yield and quality of banana production (Ploetz, 2015; Butler, 2013). Multiple studies reveal 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 focus 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 environment, including pollute soil and water and induce 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 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

75 al., 2017; Zhang et al., 2013). In our previous study, we investigated a biofertilizer

76 containing a *Bacillus* strain which isolated from rhizosphere of a continuously

77 cropped banana, we investigated its ability to promote banana growth and control

78 Fusarium wilt in banana (Shen et al., 2015; Fu et al., 2016; Fu et al., 2017). Therein,

79 we concluded that bio-organic fertilizer application is a practicable and worthy

80 measure for banana Fusarium wilt suppression.

81 Besides, crop rotation is also considered as an alternative method in soil-borne

82 disease control for its highly efficient and environmentally friendly (Krupinsky et al.,

83 2002). Crop rotation often breaks the microflora and chemical characteristics of the

84 single continuously cropped soil (Christen and Sieling, 2010; Yin et al., 2010).

85 However, rotation crop have different effects on the soil-borne diseases. The

86 mechanisms of crop rotation control soil-borne disease include inhibiting the

87 reproduction of pathogens through allelochemicals secretion, inducing the

88 antagonistic microbes against the pathogens, and improving rhizosphere microbial

89 community structure by leading different carbon into the soil through root exudates or

90 residues (Robert et al., 2014). In our previous work, banana-pineapple rotation was

91 picked out for its high-efficiency in banana Fusarium wilt disease prevention and

92 control, as well as bio-organic fertilizer application (Wang et al., 2015). However, the

93 combined control efficiencies of the two measures (pineapple-banana rotation and

94 bio-organic fertilizer application) remained unknown. Thus, there is a large need for

95 investigation of efficient disease suppression combination approaches to Fusarium

96 wilts control in banana, and hence work toward maintaining sustainable worldwide

97 industrial banana development.

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

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

100 Effective soil-borne disease suppression management strategies must demonstrate

101 significant change on the soil microbial community in addition to FOC minimization

102 (Cha et al., 2016; Chaparro et al., 2012; Gerbore et al., 2014; Mazzola and Freilich,

103 2017). We proved the effectiveness of microbial agents for bio-control by changing

104 the structure of soil microbial communities in previous reports (Fu et al., 2017; Shen

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

106 FOC population density and soil microbial community structure to attempt to explain

107 the mechanism of pineapple-banana rotation on soil borne-disease suppression (Wang

108 et al., 2015). Our results suggest that fungal community structure and several genera

109 introduced in rotation season may be the most critical factors in soil FOC decrease.

110 Unlike intercropping, *Fusarium* pathogen accumulation control through effective

111 crop rotation should be maintained longer, for at least two seasons, including rotation

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

113 banana growth cycles in our rotation pattern require long durations (almost 15 and 10

114 months, respectively, in Hainan province, China). Thus, the soil microbial community

115 structure of the original season is very important in rotation validity evaluation.

116 Furthermore, how the soil microbial community structure changes using the combined

117 control efficiencies of the two measures (pineapple-banana rotation and bio-organic

118 fertilizer application) remained unknown.

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

120 incidence fields by pineapple-banana rotation, and that control efficiency can be

121 increased when bio-control is added to the rotation. Meanwhile scheme, this will

122 concurrently change soil microbial community membership and structure. Therefore,

123 based on our previous research, we conducted field experiments to investigate the

124 effects of pineapple-banana rotation combined with biofertilizer on next season

125 banana Fusarium wilt disease suppression and soil microbial communities. Our

126 objectives follow: 1) determine the direct abilities of pineapple-banana rotation alone

and pineapple-banana rotation combined with biofertilizer application to control banana Fusarium wilt disease; 2) explore the characteristic of the soil microbial communities prompted by crop rotation and biocontrol strategies after banana harvest using the MiSeq platform; 3) evaluate the probable disease suppression mechanisms caused by our rotation and bio-control strategy.

2 Materials and Methods

2.1 Field experimental design

The field experimental site was set in Hainan Wanzhong Industrial Co., Ltd., China, a company that specializes in banana planting. The field soil had a chemical background of pH 5.12, soil organic matter (SOM) 5.57 g kg^{-1}, NH^{4+}-N 7.39mg 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 fertilizer was supplied by Lianye Bio-fertilizer Engineering Center, Ltd., Jiangsu, China, detailed information was as follows: organic fertilizer (OF), first fermentation with a 2:3 weight ratio use amino acid fertilizer and the pig manure; Bio-organic fertilizer (BIO), with a two secondarily fermented based on OF according to the solid fermentation method (Wang et al., 2013). The research was carried out in a field which a serious Fusarium wilt disease incidence (>50%) was observed after a continuously banana cropped for more than 6 years. Nine replicates in each treatment were set up with a randomized complete block design, and area of each block was 300 m^2. Banana cultivar *Musa acuminate AAA Cavendish cv*. Brazil and 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 pineapple rotation with common organic fertilizer applied in banana season (POF); (3) Banana planted after pineapple rotation treatment with bio-organic fertilizer applied (PBIO). In the rotation system, pineapple and

152 banana were planted in density of 45000 and 2,400 seedlings ha^{-1}, respectively. All

153 the organic fertilizer was applied to the soil at once as base fertilizer before banana

154 planted; other measures are 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 the all three symptoms weekly since first sick banana

159 appeared. Finally, banana wilt disease incidence was determined in the harvest time.

160 The percentage of sick plants among the total banana plants was calculated as the

161 Fusarium wilt disease incidence.

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

163 During the harvest time of last banana season, 5 healthy plants were randomly picked

164 out in each biological replicate plots for soil samples collection. Soil samples were

165 collected from four random sites at a distance of 10 cm from banana plant, a depth of

166 20 cm soil column were picked out using soil borer in each sampling site. All the 5

167 soil columns from each biological replicate plots were mixed for DNA extraction. All

168 mixed samples were placed in cold storage and transported to the laboratory. After

169 screening used a 2-mm soil sieve, total soil DNA was extracted using Clean Soil

170 DNA Isolation Kits (MoBio Laboratories Inc., Carlsbad, USA) from fine grained soil.

171 After a determination of DNA concentration and quality using NanoDrop 2000

172 (Thermo Scientific, USA), all soil DNA were diluted to a concentration of 20 ng μl^{-1}

173 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 established

181   protocols by Xiong et al. (2016). 27 cycles were performed to amplify the templates.

182   After purification, PCR products were diluted to a concentration of 10 ng μl^{-1}. of

183   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 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, the accession number was 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

202   Bray-Curtis distance metric, was carry out to evaluate the effects of rotation and

203   fertilizer type on the whole soil bacterial and fungal community by using vegan and

204   MVPART wrap package in R (version 3.2.0). In addition, to exclude the influence of

205   low abundance species, only the OTUs with average relative abundance of equal or

206   greater than 0.1% in each sample were retained (defined as retained OTUs).

207   **2.6 Network analyses**

208   Based on retained OTUs, interaction networks between OTUs were constructed using

209   the phylogenetic Molecular Ecological Network (pMEN) method according to (Zhou

210   et al., 2011) and (Deng et al., 2012). All analyses were performed using the Molecular

211   Ecological Network Analyses Pipeline (MENA). Cytoscape 2.8.2 software was used

212   to visualize the network.

213   **2.7 Statistical analysis**

214   Differences statistical analyses between the three treatments were carried out in SPSS

215   20.0 and R software. Pearson correlations among disease incidence, different Phylum

216   and FOC relative abundance were analysed in R. Linear models were also proformed

217   using R after stepwise model selection using Akaike information criteria.

218   **3 Results**

219   **3.1 Disease incidence and relative abundance of *Fusarium***

220   Pineapple rotation and biofertilizer application effectively reduced Fusarium wilt

221   disease incidence in the next season's banana plantation (**Fig. 1B**). The incidences of

222   banana Fusarium wilt disease in POF and PBIO treatments were 33.3% and 12.3%,

223   respectively, which was significantly lower than that in BOF treatment, which

224   reached up to 66.8%. The treatment, PBIO with rotation and biofertilizer application,

225   showed the lowest disease incidence with a 63.1% decrease compared with POF (**Fig.**

9

226 **1B and Table S1**). The relative abundance of *Fusarium* shows the same tendency as

227 disease incidence, and disease incidence was significantly correlated to relative

228 abundance of *Fusarium*, as revealed by MiSeq sequencing data (**Fig. 1C**).

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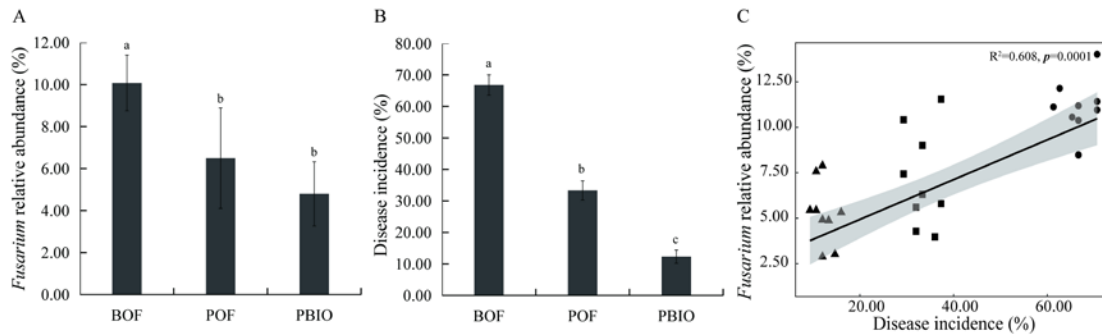
231 **Figure 1. *Fusarium* relative abundance (A), Fusarium wilt disease incidence (B) and**

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

233 abundance **(C).** BOF=Banana continuously cropped for two years with common organic

234 fertilizer applied; POF= Banana planted after Pineapple rotation with common organic

235 fertilizer applied in banana season; PBIO=Banana planted after Pineapple rotation treatment

236 with Bio-organic fertilizer applied; Bars above the histogram represent standard errors and

237 different letters indicate signifcant differences ($p < 0.05$) according to multivariate variance

238 analysis and multiple comparison results.

239 **3.2 General analyses of the high-throughput sequencing data**

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

241 and based on 97% similarity, a total of 8,346 16S and 5,647 ITS OTUs were obtained.

242 For bacteria, Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria

243 and Verrucomicrobia were the most abundant phyla, with relative abundances all

244 greater than 1%. For fungi, Ascomycota, followed by Basidiomycota,

245 Chytridiomycota, Zycomycota, and Glomeromycota were the most abundant phyla

246 (**Fig. 2**). ANOVA analysis showed that Chlamydiae, Cyanbacteria/Chloroplast,

247 Gemmatimonadetas, Nitrospirae, Planctmycetes, and Verrucomicrobia abundances

248 were significantly higher in the PBIO and POF treatment samples compared with

249 BOF treatments, besides, the relative abundance of Ascomycota was decreased in

250 PBIO treatment (Duncan test, $p < 0.05$).

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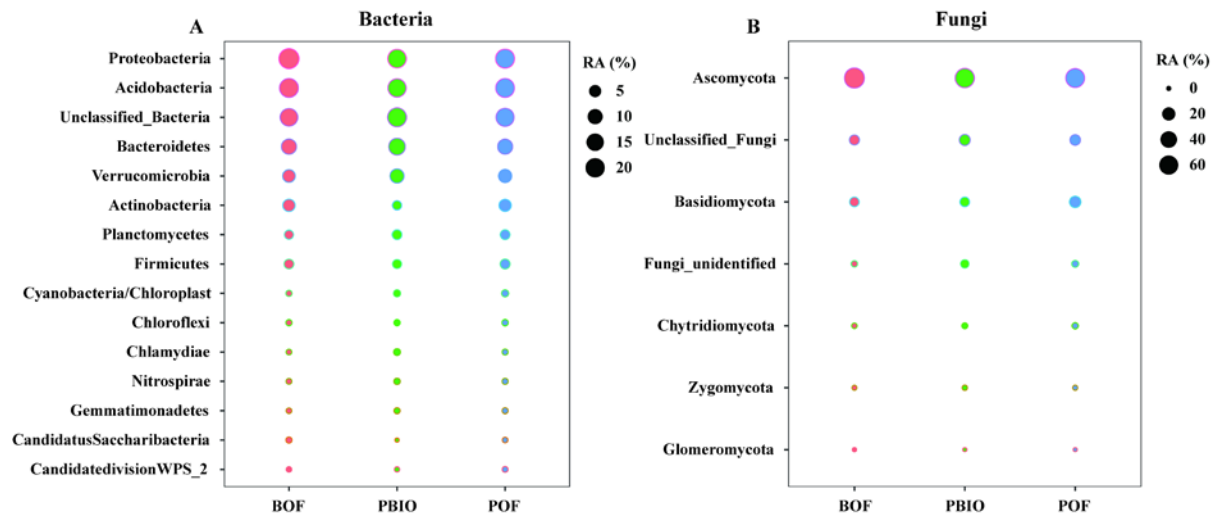
253 **Figure 2. Bubble chart of bacterial (A) and fungal (B) phyla in BOF, POF and PBIO**

254 **treatments.** BOF=Banana continuously cropped for two years with common organic

255 fertilizer applied; POF= Banana planted after Pineapple rotation with common organic

256 fertilizer applied in banana season; PBIO=Banana planted after Pineapple rotation treatment

257 with Bio-organic fertilizer applied; Values represent the average abundance across the nine

258 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 preformed to characterize α-

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

264 14,900 selected bacterial sequences and 34,943 fungal sequences. Compared with the

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

266 bacteria and fungi, and the PBIO treatment exhibited the most OTUs of all treatments

267 (**Table 1**, **Fig. S1**). Compared with the BOF treatment, pineapple-banana rotation

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

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

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

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

272 continuously cropped for two years with common organic fertilizer applied, POF=Banana

273 planted after Pineapple rotation with common organic fertilizer applied in banana season;

274 PBIO=Banana planted after Pineapple rotation treatment with Bio-organic fertilizer applied;

275 Values represent the average index of nine replicate. Means followed by the different letter

276 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

277

278   We evaluated microbial community structure by PCoA based on a UniFrac

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

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

281 from the three treatments. However, bacterial PCoA showed only two groups.

282 Unweighted UniFrac distances showed PBIO treatment separate from BOF and POF

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

284 treatment was separated from BOF treatment along the second component in fungi,

285 whereas in bacteria, POF and BOF treatments were not separate along the second

286 component (**Fig. 3A**).

287   Furthermore, MRT results indicated that Bio-fertilizer application acted the

288   largest deterministic influence on composition of both bacterial and fungal

289   community, and that cultivation was secondly important. Driven by fertilization,

290   PBIO treatment was separate from BOF and POF treatments, then, BOF and POF

291   treatments were driven by cultivation (**Fig. 3B**).

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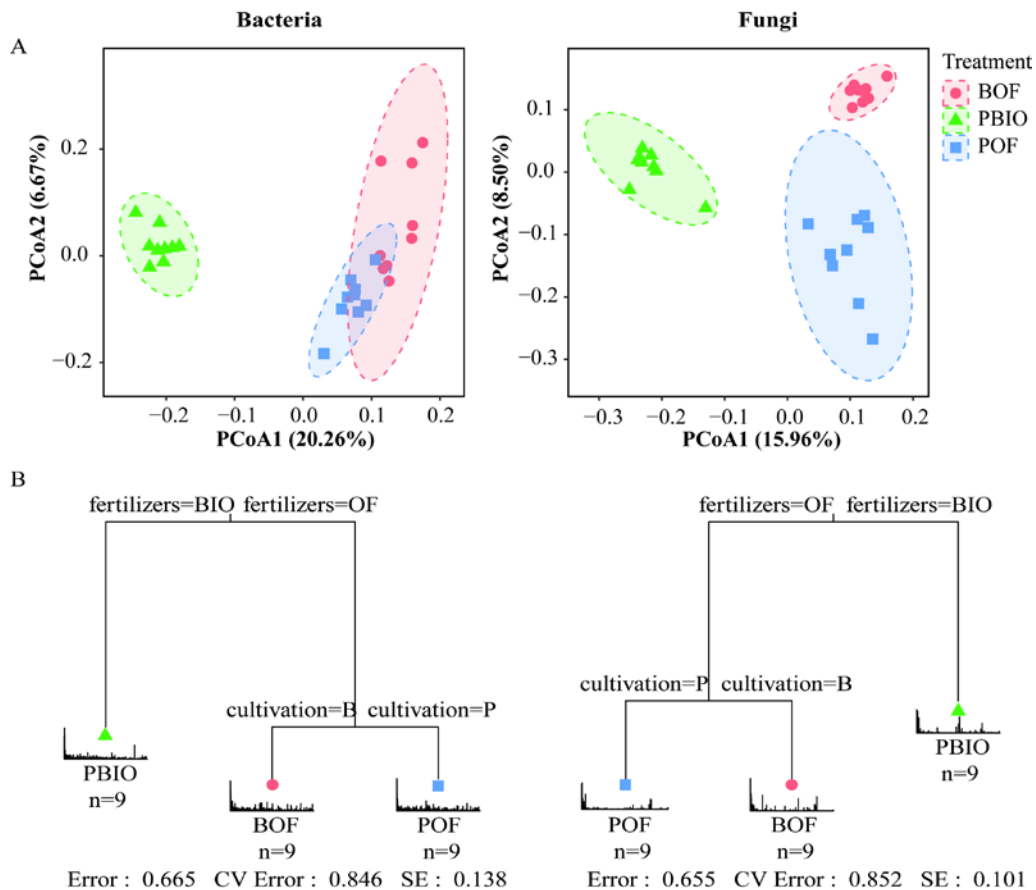
293   **Figure 3. (A) UniFrac-unweighted principle coordinate analysis of fungal and bacterial**

294   **community structures in different treatment.** BOF=Banana continuously cropped for two

295   years with common organic fertilizer applied, POF= Banana planted after Pineapple rotation

296   with common organic fertilizer applied in banana season; PBIO=Banana planted after

297   Pineapple rotation treatment with Bio-organic fertilizer applied. **(B)** Multiple regression tree

298   (MRT) analysis for the bacterial and fungal community showed the variables of fertilization

299   and cultivation in each branch.

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

Those phyla that significantly correlated with FOC abundance were selected for the evaluation of effects on soil fungal and bacterial community composition versus relative FOC abundance. Seven Bacteria phyla and three fungi phyla were significantly correlated with pathogen abundance (**Table 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**).
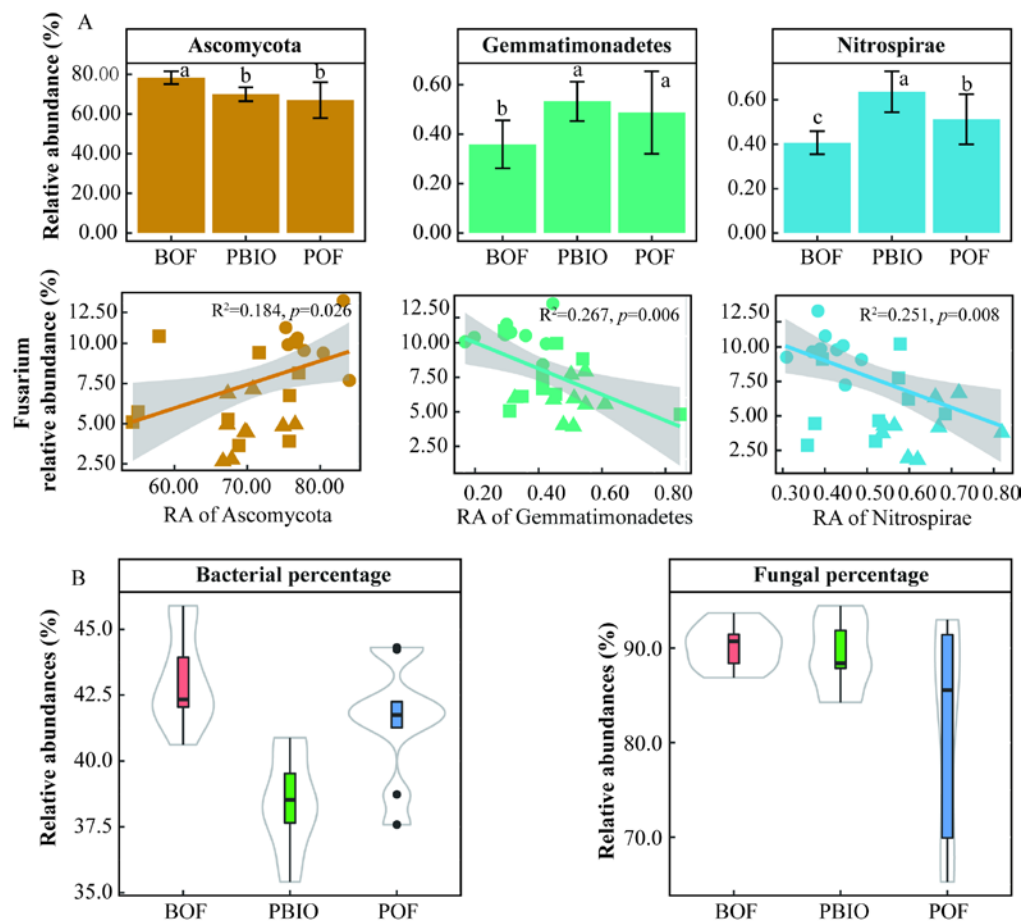

Figure 4. **(A)** The relative abundance of Ascomycota, Gemmatimonadetes and Nitrospirae phyla in the three treatments and the linear regression relationship between the Ascomycota, Gemmatimonadetes and Nitrospirae phyla and the disease incidence. **(B)** Percentage of FOC

14

related bacterial and fungal phyla in all treatments. 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 more than 0.1% relative abundance were picked out from each treatment. 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 significant correlation between two OTUs, red and green represent bacterial and fungal OTUs, respectively, and blue and red represent positive and negative correlation, respectively.
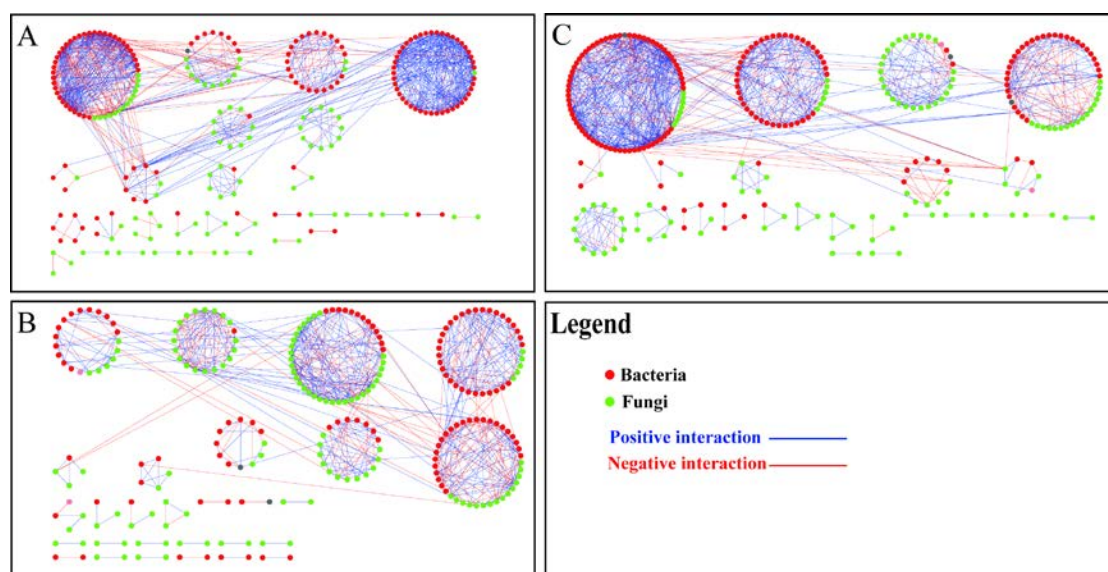
Figure 5. Network plots of bacterial and fungal communities in soil BOF (A), PBIO (B) and POF(C). BOF=Banana continuously cropped for two years with common organic fertilizer applied, POF= Banana planted after Pineapple rotation with common organic fertilizer applied in banana season; PBIO=Banana planted after Pineapple rotation treatment with Bio-organic fertilizer applied; Red nodes indicate bacteria; Green nodes indicate fungi;

330 Red lines between nodes (links) indicate negative interaction; Blue lines indicate positive

331 interaction.

332

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

334 fungal), and 241 (163 bacterial and 123 fungal) nodes were picked out from BOF,

335 PBIO, and POF treatments, respectively. F/B represents ratio of fungal to bacterial

336 nodes. The F/B ratios were 0.69, 1.01, and 0.75 in BOF, PBIO, and POF treatments,

337 respectively. This result suggests more active fungal OTUs in the PBIO treatment

338 sample, followed by POF and BOF treatments.

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

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

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

342 communities under BOF, POF and PBIO. BOF=Banana continuously cropped for two years

343 with common organic fertilizer applied, POF=Banana planted after Pineapple rotation with

344 common organic fertilizer applied in banana season; PBIO=Banana planted after Pineapple

345 rotation treatment with Bio-organic fertilizer applied; Avg K=Average connectivity; Avg

346 CC=Average clustering coefficient; GD=Average path 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

347

348   The threshold value Zi was measures the connected degree between two nodes in

349 the same module, Pi was measures the connected degree between two nodes from

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

351 divide into four categories (**Fig. 6**). Three nodes categorize as generalists (module

352 hubs) with intense connectivity to many nodes in the same modules, two from the

353 PBIO network and one from the POF network. However, no module hubs were found

354 in the BOF network. Fourteen nodes were categorized as connectors (generalists) with

355 high connectivity to several modules, eight from the BOF network and six from the

356 PBIO network. Interestingly, module hubs (generalists) were only found in pineapple-

357 banana treatment (PBIO and POF), and connectors (generalists) and module hubs

358 (generalists) were found at the same time only in pineapple-banana with bio-organic

359 fertilizer applied treatment (PBIO). Annotation information from all generalists

360 showed that bacterial OTU2 and OTU3013 belonging to *Burkholderia* were

361 generalists in PBIO network, while were absent in POF and BOF networks.

362 Additionally, another generalist OTU4869, from the PBIO network was identified as

363 *Gp6* in Acidobacteria.
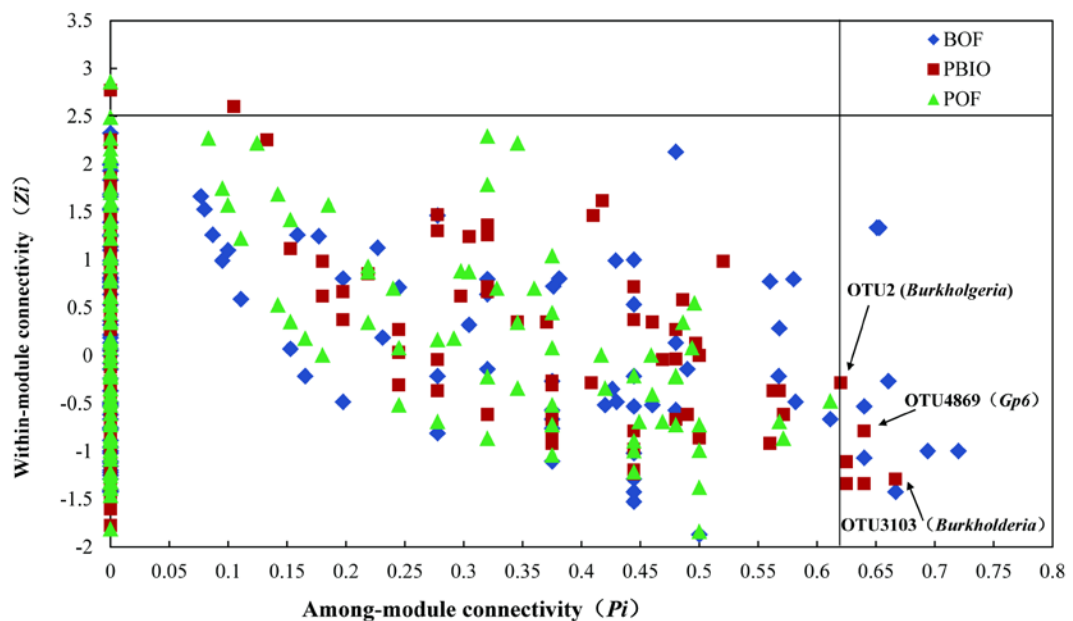
364

365 **Figure 6. *Zi-Pi* plot showing the distribution of OTUs based on their topological roles.**

366 Each symbol represents an OTU in different treatment. The threshold values of *Zi* and *Pi* for

367 categorizing OTUs were 2.5 and 0.62 respectively as proposed by Guimera and Amaral (2005)

368 and simplified by Olesen et al. (2007).

369 **3.6 Relationship between microbial indicators and incidence of banana Fusarium**

370 **wilt disease**

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

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

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

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

375 **3**).

376 **Table 3** Linear models (LM) for the relationships of microbial indicators with disease
377 incidence and the relative importance of each indicators. P was results of ANOVAs. The bold
378 value represent the *p* value is lower than 0.05 levels from the ANOVA result.

	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%				

379

380   Importantly, bacterial structure (F = 304.09, p < 0.0001, Relative Importance =

381 19.32%), fungal structure (F = 1.11, p < 0.31, Relative Importance = 16.32%), and

382 *Burkholderia* relative abundance (F = 0.76, p < 0.399, Relative Importance = 10.17%)

383 constrained disease incidence the most (with a relative importance more than 10%).

384   Besides, 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%) has significant relationship to disease

387 incidence.

388 **4 Discussion**

389 Our previous research proved the effectiveness of pineapple-banana rotation and bio-

390 organic fertilizer application were proved to be in the control of banana Fusarium wilt

391 disease (Wang et al., 2015; Fu et al., 2017). Soil microbial community change is an

392 important indicator for exploring mechanisms behind these two control measures. In

393 this 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 rotation and bio-organic fertilizer application.

396   Our previous results indicate that the pineapple-banana rotation treatments

397 significantly reduce Fusarium wilt disease incidence when compared with banana

398 monoculture. Moreover, the application of bio-organic fertilizer enhances this

399 suppression ability. Shen et al., (2018) reported that bio-fertilizer application after

400 fumigation with lime and ammonium bicarbonate was highly effectively in banana

401 Fusarium wilt disease control. Thus, although many measures can slow down the

402 spread of Fusarium wilt disease, effective control can be enhanced by the combined

403 use of more than one measure (Pda et al., 2017). So, in the current study, we explored

404 the the combined use effect of pineapple-banana rotation and bio-organic fertilizer

405   application to provide a promising strategy to manage banana Fusarium wilt disease.

406   And the results were consistent with the previous reports.

407   Significantly higher Chao1 and Faith's PD were detected in rotation and

408   biofertilizer treatment. Previous studies have shown high positive correlation between

409   disease suppression and a high diversity of bacteria with a concurrent low diversity of

410   fungi (Bonanomi et al., 2010; Fu et al., 2017). However, inconsistent with these

411   results, pineapple-banana rotation and biofertilizer treatment (PBIO) harbor a

412   significantly higher fungal richness and diversity than the other two treatments (BOF

413   and POF). This agrees with two other previous studies that indicated the importance

414   of fungal diversity in the suppressive capacity of vanilla soils and potato cropping

415   system (Xiong et al., 2017). Many previous studies have shown that the decrease of

416   soil pH is an important factor leading to soil-borne diseases. And microbial diversity

417   has been seen to increase with higher soil pH values (Liu et al., 2014; Shen et al.,

418   2013). We observed that soil pH increased in rotation and bio-organic fertilizer

419   treatment (**Table S2**), therefore, the high bacterial and fungal diversity observed in

420   our rotation and bio-organic fertilizer system may be due to high soil pH.

421   Both PCoA ordinations and MRT results reveal significant differences in

422   microbial community structure after rotation and biofertilizer applications. This is

423   supported by previous studies stating that rotation (Helena et al., 2016; Hartmann et

424   al., 2015) and bio-organic fertilizer application (Sun et al., 2015) altered soil

425   microbial community composition. Despite the apparent cultivation, MRT analysis

426   revealed fertilization effects on microbial community composition, indicating that

427   bio-organic fertilizer application in banana season was the most important factor in

428   determining microbial community composition. The result was similar to previous

429   results where bio-organic fertilizer application was the largest factor in determining

430   microbial community composition rather than temporal variability (Fu et al., 2017).

431   This is also a powerful illustration of the necessity of bio-organic fertilizer application

432   in pineapple-banana rotation.

433   Phyla level results show that rotation and biofertilizer application decreases the

434   relative abundance of Ascomycota, and increases the relative abundance of

435   Chlamydiae, Gemmatimondetes, Nitrospire, Planctomycetes, and Verrucomicrobia,

436   which are all associated with disease suppression in previous reports (Trivedi et al.,

437   2017; Shen et al., 2018). Our fungal result is consistent with previous observations of

438   low Ascomycota phylum abundance in suppressive soil, which is logical because

439   Ascomycetes constitutes the largest group of soil pathogens (Lu et al., 2013).

440   Furthermore, this tendency was observed in our previous report in which a decrease of

441   Ascomycetes was considered as important factors in FOC decrease during the

442   pineapple season (Wang et al., 2015). The bacterial result is partly consistent with

443   previous observations that rotations with wild rocket and Indian mustard increase the

444   *Nitrospira* and Gemmatimonadetes content (Jin et al., 2019). It's worth noting that our

445   BIO was secondary fermentation with *Bacillus* added, while, *Bacillus* genus was not

446   enriched in the BIO treatment soil. Moreover, microbial structure appeared to be the

447   most constrained factor with disease incidence in linear models between microbial

448   indicators and the incidence of banana Fusarium wilt disease. Xiong et al (2017)

449   suggest that microbial species introduced by biofertilizer application induce wilt

450   suppression by microbiome transform, rather than pathogen suppression directly.

451   Alteration of the soil microbiome may cause more response than the added *Bacillus*

452   itself in the PBIO treatment sample in our case as well.

453   We earlier confirmed that pineapple-banana rotation reduces the amount of

454   *Fusarium oxysporum* mainly by modulating fungal communities during pineapple

455   season (Wang et al., 2015). In the present research, compared with bacteria, a higher

456   percentage of FOC-correlation genus in fungi was observed in all treatments. Even

457 though more kinds of bacteria are related to FOC, more percentage of fungi shows

458 relevance. These results agree with findings of Mona et al. (2014) and Cai et al.

459 (2017), who reported that fungal communities have a more crucial response to soil

460 factors changes than bacterial communities. It is worth noting that fungal

461 communities were more dissimilar between the Pineapple-banana rotation and Maize-

462 banana rotation treatments than bacteria in our previous studies (Wang et al., 2015).

463 Thus, the higher FOC-relevance found in fungal community both in the pineapple and

464 banana season further reinforced the importance of fungal community changes in our

465 case.

466   Several researchers have used microbial molecular ecological networks to study

467 complex microbial ecological systems in suppressive soils, including corn-potato

468 rotations (Lu et al., 2013) and vanilla (Xiong et al., 2017). We found microbial

469 molecular ecological networks to reveal distinct differences between the microbial

470 communities associated with the three treatments in our research. More fungal OTUs

471 were picked up in the PBIO treatment samples, followed by the POF and BOF

472 treatments, based on the F/B ratio. Although the OTUs selected to build the network

473 are only a part of the whole system, there is no doubt that these OTUs are very

474 important for soil function (Coyte et al., 2015). Therefore, we conclude that the large

475 number of fungal OTUs present in the system may lead to changes in soil function.

476 PBIO, POF, and BOF soils harbored modules with modularity values of 0.718, 0.642,

477 and 0.616, respectively, in this study. Modularity represents how well the network

478 was organized (Zhou et al., 2011). Thus, PBIO network, which possess high

479 modularity, has more connections between nodes in the same modules, followed by

480 POF and BOF. The altered networks compared with POF and BOF may partially

481 contribute latent attributes to higher disease suppression in our rotation and bio-

482 organic fertilizer application trials. Furthermore, no module hubs (generalists) are

483   present in the BOF network, whereas all three module hubs are found in the

484   pineapple-banana rotation network as *Zi-Pi* relationship indicated. In all three

485   networks, connectors (generalists) and module hubs (generalists) were found at the

486   same time only with the PBIO treatment. Generalists typically only occupy a small

487   fraction of a community; however, the presence of those generalists is quite important

488   (Zhou et al., 2011; Jens et al., 2011). These nodes could enhance connecters within or

489   among modules. If the network is poorly connected, or not connected at all, the

490   community is predicted to be disordered and fluxes of energy, material, and

491   information would not be efficient (Lu et al., 2013). So in our case, these generalists

492   found in PBIO suggest that the microbial community structure was more orderly and

493   powerful than the other two treatments.

494   Annotation information from all the generalists found in our study show that

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

496   PBIO network, but were not observed in the POF and BOF networks.

497   Correspondingly, a high abundance of *Burkholderia* and high percentage of

498   antagonistic *Burkholderia* was found during pineapple season in our previous report

499   (Wang et al., 2015). In addition, our linear model analysis shows that besides bacterial

500   and fungal structure, *Burkholderia* relative abundance constrains disease incidence

501   with the high Relative Importance factor of 10.17%. *Burkholderia* genus was versatile

502   organisms for its powerful function of occupying ecological niche and variety

503   functions include biological control and plant growth promotion and so on in

504   agriculture (Coenye and Vandamme, 2003). This suggests that even though the

505   relative abundance of *Burkholderia* in PBIO was not that high, it is the change of

506   network structure in the rotation and bio-organic fertilizer treatments that attribute to

507   the general wilt suppression activity, and that change may specifically be due to

508   special functions of *Burkholderia*. Additionally, one generalist in the PBIO treatment

509 sample was identified as *Gp6* in Acidobacteria. Although no Acidobacteria

510 antimicrobial activities have previously been recorded, several studies have

511 demonstrated the Acidobacteria is greatly affected by soil pH and *Gp6* was positively

512 correlated with soil pH (Bartram et al., 2014; Jones et al., 2009). Therefore, the

513 special function of *Gp6* in PBIO network probably results from an increase in soil pH.

514 **5 Conclusions**

515 An expansion of previous work, this study revealed that pineapple-banana rotation

516 combined with bio-organic fertilizer application during the banana season is effective

517 in reducing *Fusarium spp*. abundance and banana Fusarium wilt. Several different

518 analyses indicate that bacterial and fungal communities, especially fungal structure,

519 are changed by rotation and bio-organic fertilizer application. Bio-organic fertilizer

520 inhibits Fusarium wilt disease by changing the soil microbial structure, rather than

521 any designated microorganism. Large changes in the fungal community and special

522 *Burkholderia* functions in the network are likely the most responsible factors for soil

523 borne-disease suppression. Pineapple-banana rotation combined with bio-organic

524 fertilizer application has strong potential for the sustainable management of banana

525 Fusarium wilt disease.

526 **Data availability**

527 All data are available. The sequencing data have been submitted to the NCBI

528 Sequence Read Archive database (SRP234066).

529 **Author contributions**

530 Rong Li and Beibei Wang designed the research and wrote the manuscript. Beibei

531 Wang, YannanOu and ZongzhuanShen performed trials and conducted fieldwork.

532 Beibei Wang and Jinming Yang analyzed the data. Rong Li, Lin Fu, Yunze Ruan,

533 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

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