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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24 **Abstract**

25 Crop rotation and bio-organic fertilizer application have historically been employed as

26 efficient management strategies for soil-borne disease suppression through soil

27 microbiome manipulation. However, details of how this occurs, and to what extent the

28 combination of methods affects soil microbiota reconstruction from diseased soils

29 lacks investigation. In this study, pineapple-banana rotation combined with

30 biofertilizer application was used to suppress banana *Fusarium* wilt disease, and

31 effects on both bacterial and fungal communities were investigated using the Miseq

32 Illumine sequencing platform. Our results show that pineapple-banana rotation

33 significantly reduces *Fusarium* wilt disease incidence, and that the application of bio-

34 organic fertilizer causes additional suppression. Bacterial and fungal communities

35 thrive using rotation in combination with bio-organic fertilizer application: taxonomic

36 and phylogenetic α-diversity in both bacteria and fungi increase along with disease

37 suppression. Between the two strategies, bio-organic fertilizer application affects both

38 bacterial and fungal community composition most predominantly, followed by

39 rotation. Large-scale changes in the fungal community composition and special

40 *Burkholderia*-related network functions contribute to the observed soil borne-disease

41 suppression. Our results indicate that pineapple-banana rotation combined with bio-

42 organic fertilizer application has strong potential for the sustainable management of

43 banana *Fusarium* wilt disease.

44

45 **Keywords:** Banana *Fusarium* wilt; Pineapple-banana rotation; Biofertilizer; Disease

46 suppression; Microbiome structure

47

48

1 Introduction

Banana *Fusarium* wilt disease, which caused by *Fusarium oxysporum* f. sp. *cubense race 4* (FOC) forms a major constraint for 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 was used in fields with high incidence rates, and bio-control was 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 have focused 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 was 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 was 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 was a practicable and worthy

80 measure for banana *Fusarium* wilt suppression.

81   Besides, crop rotation was 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

127   and pineapple-banana rotation combined with biofertilizer application to control

128   banana *Fusarium* wilt disease; 2) explore the characteristic of the soil microbial

129   communities prompted by crop rotation and biocontrol strategies after banana harvest

130   using the MiSeq platform; 3) evaluate the probable disease suppression mechanisms

131   caused by our rotation and bio-control strategy.

132   **2 Materials and Methods**

133   **2.1 Field experimental design**

134   The field experimental site was set in Hainan Wanzhong Industrial Co., Ltd., China, a

135   company that specializes in banana planting. The field soil had a chemical

136   background of pH 5.12, soil organic matter (SOM) 5.57 g kg^{-1}, NH^{4+}-N 7.39mg kg^{-1},

137   NO_3-N 6.68 mg kg^{-1}, available P 56.9 mg kg^{-1}a and available K 176.4 mg kg^{-1}. The

138   fertilizer was supplied by Lianye Bio-fertilizer Engineering Center, Ltd., Jiangsu,

139   China, detailed information was as follows: organic fertilizer (OF), first fermentation

140   with a 2:3 weight ratio use amino acid fertilizer and the pig manure; Bio-organic

141   fertilizer (BIO), with a two secondarily fermented based on OF according to the solid

142   fermentation method (Wang et al., 2013). The research was carried out in a field

143   which a serious *Fusarium* wilt disease incidence (>50%) was observed after a

144   continuously banana cropped for more than 6 years. Nine replicates in each treatment

145   were set up with a randomized complete block design, and area of each block was 300

146   m^2. Three treatments were assigned: (1) banana continuously cropped for two years

147   with common organic fertilizer applied (BOF); (2) banana planted after pineapple

148   rotation with common organic fertilizer applied in banana season (POF); (3) Banana

149   planted after pineapple rotation treatment with bio-organic fertilizer applied (PBIO).

150   In the rotation system, pineapple and banana were planted in density of 45000 and

151   2,400 seedlings ha^{-1}, respectively. All the organic fertilizer was applied to the soil at

152   once as base fertilizer before banana planted; other measures are consistent with

153   common banana production.

2.2 Banana *Fusarium* wilt disease incidence statistics

155   Old leaves yellowing, stem crack and new leaves diminishing were the three typical

156   wilt symptoms of banana *Fusarium* wilt disease. Disease incidence was calculated

157   based on the appearance of the all three symptoms weekly since first sick banana

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

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

160   *Fusarium* wilt disease incidence.

2.3 Soil sample collection and DNA extraction

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

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

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

165   20 cm soil column were picked out using soil borer in each sampling site. All the 5

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

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

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

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

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

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

172   for PCR amplification.

2.4 Polymerase chain reaction amplification and Illumina Miseq sequencing

174   Primers   F520   (5'-AYTGGGYDTAAAGNG-3')   and   R802   (5'-

175   TACNVGGGTATCTAATCC-3') were chosen to amplify the V4 regions of 16 S

176   rRNA gene (Claesson et al., 2009). Primers ITS (5'-GGA AGT AAA AGT CGT

177 AAC AAG G-3') and ITS (5'-TCC TCC GCT TAT TGA TAT GC-3') were chosen

178 for amplification of the fungal ITS region (Schoch et al., 2012).

179 PCR reactions for each sample were performed according to established

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

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

182 Fungal and bacterial PCR products sequencing were performed on the Illumina

183 MiSeq platform of Personal Biological Co., Ltd (Shanghai, China).

184 **2.5 Bioinformatic analysis**

185 Raw sequences were separated based on the unique 6-bp barcode and sheared of the

186 adaptor and primer using QIIME (Caporaso et al., 2010). Forward and reverse

187 sequences were merged after removal of low-quality sequences. Then, the merged

188 sequences were processed to build the operational taxonomic unit (OTU) at an

189 identity level of 97% according to the UPARSE pipeline. Next, representative

190 sequences of each OTU were classified in the RDP and UNITE databases for bacteria

191 and fungi, respectively (Edgar, 2013; Wang et al., 2007). All raw sequences were

192 deposited in NCBI, the accession number was SRP234066.

193 To compare the relative levels of OTU diversity across all samples, a rarefaction

194 curve was formed using Mothur software (Schloss et al., 2009). The fungal and

195 bacterial diversity was estimated using phylogenetic diversity (PD) indices and Chao1

196 richness which were also calculated based on neighbor-joining phylogenetic trees

197 generated using Mothur pipeline (Faith, 1992).

198 To compare bacterial and fungal community structures among all soil samples,

199 principal coordinate analysis (PCoA) was set up based on the weighted UniFrac

200 metric matrix (Lozupone et al., 2005). Multiple regression tree (MRT), based on

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

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

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

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

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

206 **2.6 Network analyses**

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

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

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

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

211 to visualize the network.

212 **2.7 Statistical analysis**

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

214 20.0 and R software. Pearson correlations among disease incidence, different Phylum

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

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

217 **3 Results**

218 **3.1 Disease incidence and relative abundance of *Fusarium***

219 Pineapple rotation and biofertilizer application effectively reduced *Fusarium* wilt

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

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

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

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

224 showed the lowest disease incidence with a 63.1% decrease compared with POF. The

225 relative abundance of *Fusarium* shows the same tendency as disease incidence, and

226   disease incidence was significantly correlated to relative abundance of *Fusarium*, as

227   revealed by MiSeq sequencing data (**Fig. 1C**).

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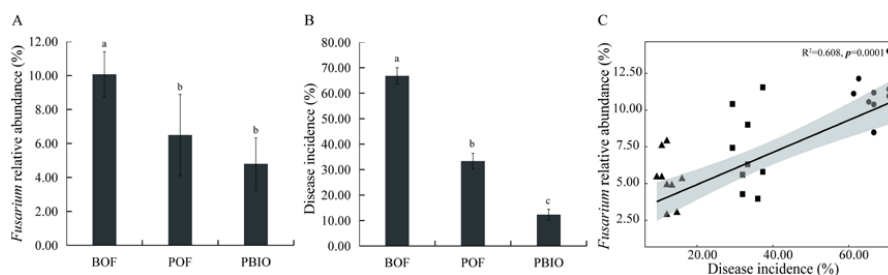
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230   **Figure 1.** *Fusarium* **wilt disease incidence,** *Fusarium* **relative abundance and Pearson**

231   **correlations between them. (A and B)** *Fusarium* wilt disease incidence and *Fusarium*

232   relative abundance for banana continuously cropped for two years with common organic

233   fertilizer applied (BOF), banana planted after Pineapple rotation with common organic

234   fertilizer applied in banana season (POF); banana planted after Pineapple rotation treatment

235   with Bio-organic fertilizer applied (PBIO). Bars above the histogram represent standard errors

236   and different letters indicate signifcant differences ($p < 0.05$) according to multivariate

237   variance analysis and multiple comparison results. **(C)** Pearson correlations between

238   *Fusarium* wilt disease incidence and *Fusarium* relative abundance.

239

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 OTUs were obtained.

243   For bacteria, *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*,

244   *Proteobacteria* and *Verrucomicrobia* were the most abundant phyla, with relative

245   abundances all greater than 1%. For fungi, *Ascomycota*, followed by *Basidiomycota*,

246   *Chytridiomycota*, *Zycomycota*, and *Glomeromycota* were the most abundant phyla

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

248   *Gemmatimonadetas*, *Nitrospirae*, *Planctmycetes*, and *Verrucomicrobia* abundances

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

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

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

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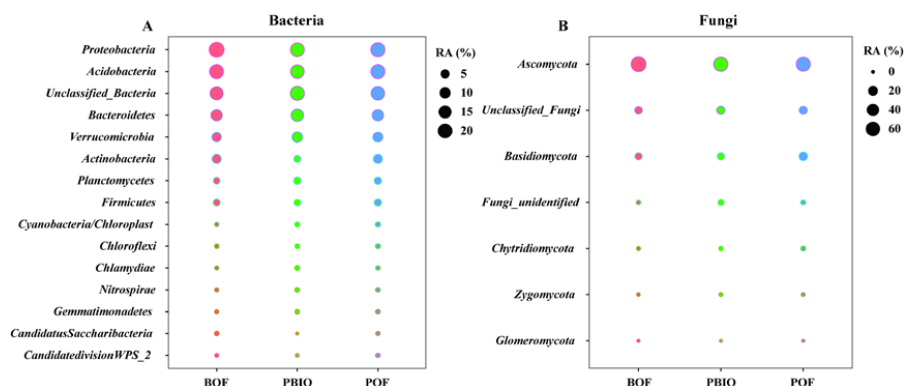
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255   **Figure 2. Bubble chart of bacterial (A) and fungal (B) phyla in BOF, POF and PBIO**

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

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

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

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

260   replicate libraries for soil samples collected from each treatment.

261

262   **3.3 Effect of pineapple rotation and biofertilizer application on soil microbial**

263   **diversity and community structure**

264   Rarefaction analyses, Chao1 and Faith's PD were preformed to characterize α-

265   diversity. Rarefaction analyses show that the number of OTUs tends to smooth at

266   14,900 selected bacterial sequences and 34,943 fungal sequences. Compared with the

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

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

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

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

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

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

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

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

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

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

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

278   for a given factor are significantly different ($p < 0.05$; Duncan test).

	Treatment	Numbers of Otus	Chao1	Faith's PD
Bacteria	BOF	2606±071 b	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

279

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

281   weighted distance matrix to analyze differences of community composition in three

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

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

284   Weighted UniFrac distances showed PBIO treatment separate from BOF and POF

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

286   treatment was separate from BOF treatment along the second component in fungi,

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

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

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

290   largest deterministic influence on composition of both bacterial and fungal

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

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

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

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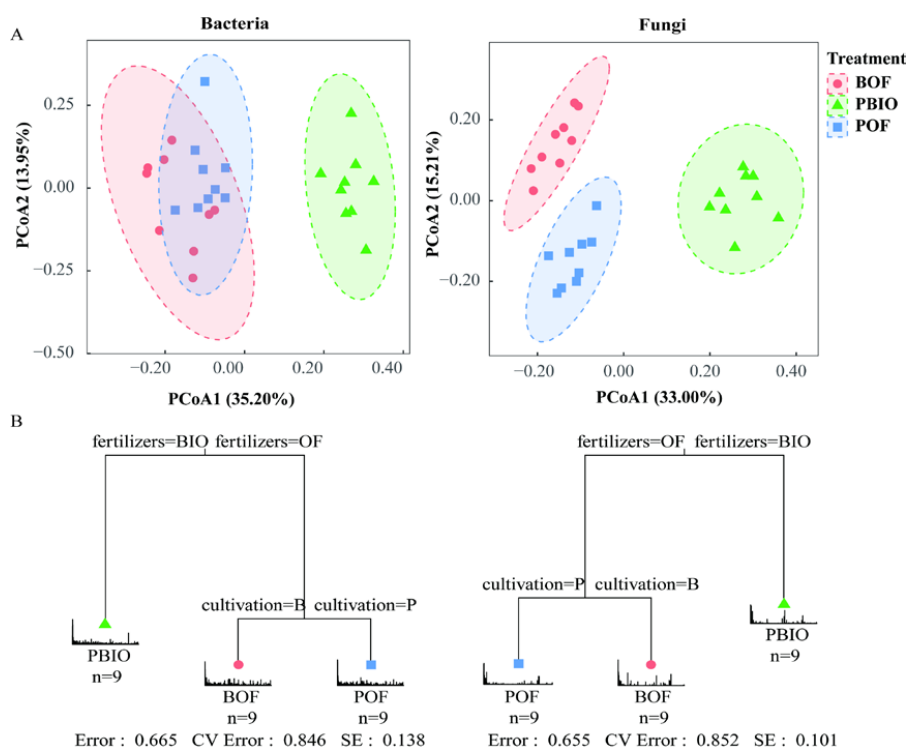

295   **Figure 3. (A) UniFrac-weighted principle coordinate analysis of fungal and bacterial**

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

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

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

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

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

301   and cultivation in each branch.

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

bacterial community composition

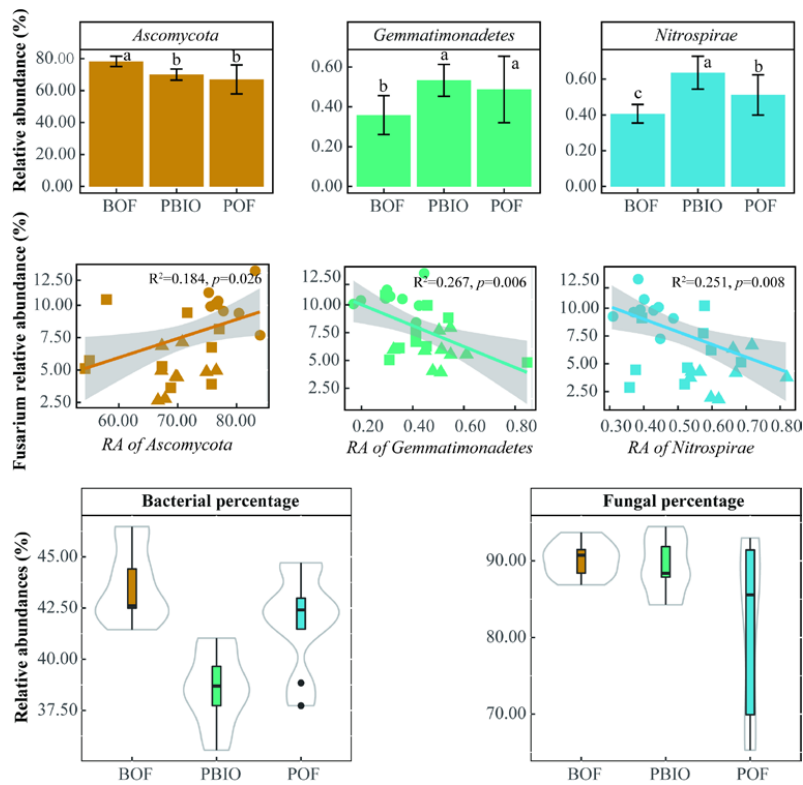
304   Those phyla that significantly correlated with FOC abundance were selected for the

305   evaluation of effects on soil fungal and bacterial community composition versus

306   relative FOC abundance. Eight Bacteria phyla and three fungi phyla were

307   significantly correlated with pathogen abundance (**Table S2 and S3**). Moreover, more

308   fungi were significantly correlated with FOC abundance compared with bacteria,

309   based on the percentage of FOC related phyla showing this trend (**Fig. 4B**).

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311   **Figure 4. (A)**The relative abundance of *Ascomycota, Gemmatimonadetes* and *Nitrospirae*

312   phyla in the three treatments and the linear regression relationship between the *Ascomycota,*

313   *Gemmatimonadetes* and *Nitrospirae* phyla and the disease incidence. **(B)** Percentage of FOC

314   related bacterial and fungal phyla in all treatments. Different letters above the bars indicate a

315   significant difference at the 0.05 probability level according to the Duncan test.

316   **3.5 Key topological properties of the networks**

317   We built networks to show interactions among genera in the different treatments;

318   those OTUs with a more than 0.1% relative abundance were picked out from each

319   treatment. 301 OTUs were selected from the BOF treatment (122 bacterial and 179

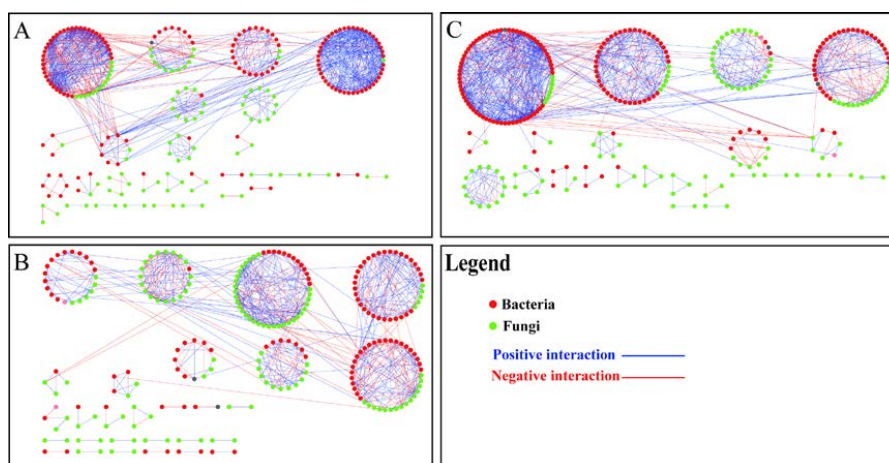
320   fungal), 323 OTUs were selected from the PBIO treatment (152 bacterial and 171

321   fungal), and 324 OTUs were selected from the POF treatment (140 bacterial and 184

322   fungal). Random matrix theory was used to build the networks. As shown in **Fig. 5**,

323   each node represents an OTU, each link shows significant correlation between two

324   OTUs, red and green represent bacterial and fungal OTUs, respectively, and blue and

325   red represent positive and negative correlation, respectively.

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

328   **and POF(C).** BOF=Banana continuously cropped for two years with common organic

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

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

331   with Bio-organic fertilizer applied; Red nodes indicate bacteria; Green nodes indicate fungi;

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

333   interaction.

334

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

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

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

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

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

340   sample, followed by POF and BOF treatments.

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

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

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

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

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

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

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

348   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

349

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

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

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

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

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

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

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

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

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

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

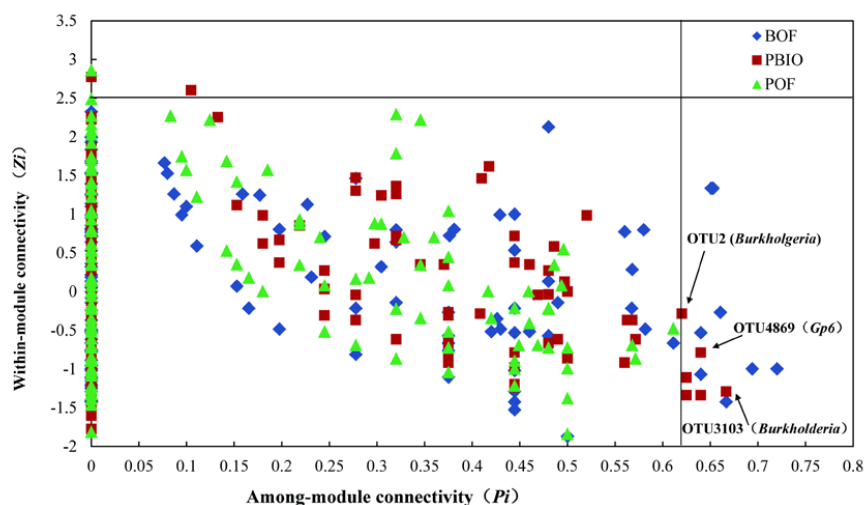
360   (generalists) were found at the same time only in pineapple-banana with bio-organic

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

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

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

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

365   *Gp6* in *Acidobacteria*.

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

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

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

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

371 **3.6 Relationship between microbial indicators and incidence of banana *Fusarium***

372 **wilt disease**

373 Bacterial and fungal structure (weighted PCoA1), richness (Chao1), and Faith's PD;

374 *Ascomycota*, *Gemmatimonadetes*, and *Nitrospirae* phyla relative abundances; and

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

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

377 **3**).

378 **Table 3** Linear models (LM) for the relationships of microbial indicators with disease
379 incidence and the relative importance of each indicators. P was results of ANOVAs. *p < 0.05,
380 **p < 0.01 and ***p < 0.001.

	df	F	*P*	Relative Importance
Bac-PCoA1	1	276.19	<0.001***	15.76%
Fun-PCoA1	1	11.44	0.004**	17.80%
Bac-Chao1	1	7.38	0.017*	9.50%
Fun-Chao1	1	0.36	0.559	4.05%
Bac-Faith's PD	1	1.92	0.188	6.72%
Fun-Faith's PD	1	1.16	0.300	6.33%
Ascomycota	1	14.21	0.002**	3.29%
Fusarium Relative abundance	1	0.36	0.557	8.06%
Nitrospirae	1	0.05	0.820	7.50%
Gemmatimonadetes	1	0.10	0.752	2.81%
Burkholderia	1	0.41	0.534	10.40%
Bacillus	1	1.74	0.209	3.52%
Residuals	14			
Model summary: R^2=0.944, AIC =-122.98, p < 0.001				
Total response variance: 95.74%				

381

382   Importantly, bacterial structure (F = 276.19, p < 0.001, Relative Importance =

383   15.76%), fungal structure (F = 11.44, p < 0.004, Relative Importance = 17.80%), and

384   *Burkholderia* relative abundance (F = 0.41, p < 0.534, Relative Importance = 10.40%)

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

386   Besides, based on linear regression analyses between disease incidence and

387   selected microbial indicators, we found that bacterial structure (F = 276.19, p <0.001,

388   Relative Importance = 15.76%), fungal structure (F = 11.44, p = 0.004, Relative

389   Importance = 17.80%), bacterial richness (F = 7.38, p = 0.017, Relative Importance =

390   9.50%), and *Ascomycota* relative abundance (F = 14.21, p = 0.002, Relative

391   Importance = 3.29%) have significant relationship to disease incidence.

392   **4 Discussion**

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

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

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

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

397   this study, disease incidence and soil microbial community characteristics during the

398   banana-growing season were measured to evaluate the control effect and potential

399   impact of rotation and bio-organic fertilizer application.

400   Our previous results indicate that two pineapple-banana rotation treatments

401   significantly reduce *Fusarium* wilt disease incidence and the relative abundance of

402   *Fusarium*, compared with banana monoculture. Moreover, the application of bio-

403   organic fertilizer enhances this suppression ability. (Shen et al., 2018) reported that

404   bio-fertilizer application after fumigation with lime and ammonium bicarbonate was

405   highly effectively in banana *Fusarium* wilt disease control. Thus, although many

406   measures can slow down the spread of *Fusarium* wilt disease (Pda et al., 2017),

407   effective control can be enhanced by the combined use of more than one measure.

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

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

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

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

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

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

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

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

416   system (Xiong et al., 2017). Microbial diversity has been seen to increase with higher

417   soil pH values (Liu et al., 2014; Shen et al., 2013). We observed that soil pH

418   increased in rotation and bio-organic fertilizer treatment (**Table S1**), therefore, the

419   high bacterial and fungal diversity observed in our rotation and bio-organic fertilizer

420   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 we observed that in all treatments,

456 compared with bacteria, more fungi significantly correlate with FOC abundance. 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. Thus, the higher FOC-relevance found in

461   fungal community both in the pineapple and banana season further reinforced the

462   importance of fungal community changes in our case.

463   We found microbial molecular ecological networks to reveal distinct differences

464   between the microbial communities associated with the three treatments. More fungal

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

466   treatments, based on the F/B ratio. Several researchers have used microbial molecular

467   ecological networks to study complex microbial ecological systems in suppressive

468   soils, including corn-potato rotations (Lu et al., 2013) and vanilla (Xiong et al., 2017).

469   Although the OTUs selected to build the network are only a part of the whole system,

470   there is no doubt that these OTUs are very important for soil function (Coyte et al.,

471   2015). Therefore, we conclude that the large number of fungal OTUs present in the

472   system may lead to changes in soil function. PBIO, POF, and BOF soils harbored

473   modules with modularity values of 0.718, 0.642, and 0.616, respectively, in this study.

474   Modularity represents how well the network was organized (Zhou et al., 2011). Thus,

475   PBIO network, which possess high modularity, has more connections between nodes

476   in the same modules, followed by POF and BOF. The altered networks compared

477   with POF and BOF may partially contribute latent attributes to higher disease

478   suppression in our rotation and bio-organic fertilizer application trials. Furthermore,

479   no module hubs (generalists) are present in the BOF network, whereas all three

480   module hubs are found in the pineapple-banana rotation network as *Zi-Pi* relationship

481   indicated. In all three networks, connectors (generalists) and module hubs (generalists)

482   were found at the same time only with the PBIO treatment. Generalists typically only

483   occupy a small fraction of a community; however, the presence of those generalists is

484   quite important (Zhou et al., 2011; Jens et al., 2011). These nodes could enhance

485   connecters within or among modules. If the network is poorly connected, or not

486   connected at all, the community is predicted to be disordered and fluxes of energy,

487   material, and information would not be efficient (Lu et al., 2013). So in our case,

488   these generalists found in PBIO suggest that the microbial community structure was

489   more orderly and powerful than the other two treatments.

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

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

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

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

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

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

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

497   with the high Relative Importance factor of 10.40%. *Burkholderia* genus was versatile

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

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

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

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

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

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

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

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

506   antimicrobial activities have previously been recorded, several studies have

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

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

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

510 **5 Conclusions**

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

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

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

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

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

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

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

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

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

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

521 *Fusarium* wilt disease.

522 **Data availability**

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

524 Sequence Read Archive database (SRP234066).

525 **Author contributions**

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

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

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

529 Yan Zhao and Qirong Shen participated in the design of the study, provided

530 comments and edited the manuscript. All authors read and approved the final

531 manuscript.

532 **Competing interests**

533   The authors declare that the research was conducted in the absence of any commercial

534   or financial relationships that could be construed as a potential conflict of interest.

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