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

Beibei Wang[1], Jinming Yang[1], Zongzhuan Shen[2], Yannan Ou[2], Lin Fu[3], Yan Zhao[1], Rong Li[2*], Yunze Ruan[1], Qirong Shen[2]

[1]Hainan key Laboratory for Sustainable Utilization of Tropical Bio-resources, College of tropical crops, Hainan University, 570228 Haikou, China

[2]Jiangsu Provincial Key Lab for Solid Organic Waste Utilization, National Engineering Research Center for Organic-based Fertilizers, Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, Nanjing Agricultural University, Nanjing, 210095, P.R. China

[3] School of Life Science, Liaoning Universities, 110036, Shenyang, China

Corresponding author: Rong Li(lirong@njau.edu.cn)

Abstract

Crop rotation and bio-organic fertilizer application have historically been employed as efficient management strategies for soil-borne disease suppression through soil microbiome manipulation. However, details of how this occurs and to what extent the combination of methods affects soil microbiota reconstruction from diseased soils lack investigation. In this study, pineapple-banana rotation combined with biofertilizer application was used to suppress banana Fusarium wilt disease, and the effects on both bacterial and fungal communities were investigated using the MiSeq Illumine sequencing platform. Our results showed that pineapple-banana rotation significantly reduced Fusarium wilt disease incidence, and that the application of bio-organic fertilizer caused additional suppression. Bacterial and fungal communities thrived using rotation in combination with bio-organic fertilizer application: taxonomic and phylogenetic α-diversity in both bacteria and fungi increased along with disease suppression. Between the two strategies, bio-organic fertilizer application affected both bacterial and fungal community composition most predominantly, followed by rotation. Large-scale changes in the fungal community composition and special *Burkholderia*-related network contributed to the observed soil-borne disease suppression. Our results indicated that pineapple-banana rotation combined with bio-organic fertilizer application has strong potential for the sustainable management of banana Fusarium wilt disease.

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

1 Introduction

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

Of all available management strategies, chemical pesticides are optimally effective against soil-borne plant pathogens, but this strategy is not friendly to the environment, including polluting soil and water and inducing the emergence of drug-resistant strains (Le et al., 2016). Biological control using beneficial soil microorganisms such as *Bacillus* and *Trichoderma* against soil-borne pathogens is considered a sustainable alternative to chemical pesticides (Alabouvette et al., 2009; Fravel et al., 2003; Qiu et al., 2012). Biofertilizers combine the advantage of introducing beneficial microbes that will occupy niches with the inclusion of organic material that will create additional niches for beneficial indigenous microbes (Cai et

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

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

77 banana, and 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). Therefore,

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

80 measure for banana Fusarium wilt suppression.

81 In addition, crop rotation is also considered an alternative method in soil-borne

82 disease control because it is highly efficient and environmentally friendly (Krupinsky

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

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

85 However, crop rotation has different effects on soil-borne diseases. The mechanisms

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

87 pathogens through allelochemical secretion, inducing antagonistic microbes against

88 pathogens, and improving rhizosphere microbial community structure by introducing

89 different carbon compounds into the soil through root exudates or residues (Robert et

90 al., 2014). In our previous work, banana-pineapple rotation was selected for its high-

91 efficiency in banana Fusarium wilt disease prevention and control, as well as bio-

92 organic fertilizer application (Wang et al., 2015). However, the combined control

93 efficiencies of the two measures (pineapple-banana rotation and bio-organic fertilizer

94 application) remain unknown. Thus, there is a great need to investigate efficient

95 disease suppression-combined approaches for Fusarium wilt control in banana and

96 hence to work towards maintaining sustainable worldwide industrial banana

97 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 changes in 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 biocontrol by changing the

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

105 2015). We also investigated the influences of quarterly rotation (pineapple) on FOC

106 population density and soil microbial community structure while attempting to

107 explain the mechanisms of pineapple-banana rotation on soil borne-disease

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

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

110 critical factors in soil FOC decrease.

111   Unlike intercropping, controlling *Fusarium* pathogen accumulation through

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

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

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

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

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

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

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

119 rotation and bio-organic fertilizer application) remains unknown.

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

121 incidence fields by pineapple-banana rotation and that the control efficiency can be

122 increased when biocontrol is added to the rotation. In addition, this scheme will

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

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

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

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

127 objectives were to 1) determine the direct effects of pineapple-banana rotation alone

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

129 banana Fusarium wilt disease; 2) explore the characteristics of the soil microbial

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

131 using the MiSeq platform; and 3) evaluate the probable disease suppression

132 mechanisms caused by our rotation and biocontrol strategy.

133 **2 Materials and Methods**

134 **2.1 Field experimental design**

135 The field experimental site was set at Hainan Wanzhong Industrial Co., Ltd., China, a

136 company that specialized in banana planting during December 2011and June 2014.

137 The field soil had a chemical background of pH 5.12, soil organic matter (SOM) 5.57

138 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

139 available K 176.4 mg kg^{-1}. Fertilizer was supplied by Lianye Biofertilizer Engineering

140 Center, Ltd., Jiangsu, China. The organic fertilizer (OF) used in our study was a first

141 fermentation with a 2:3 weight ratio using amino acid fertilizer and pig manure. The

142 bio-organic fertilizer (BIO) was a secondarily fermentation based on OF according to

143 the solid fermentation method (Wang et al., 2013). The research was carried out in a

144 field in which a serious Fusarium wilt disease incidence (>50%) was observed after a

145 continuous banana cropping for more than 6 years. Nine replicates in each treatment

146 were set up with a randomized complete block design, and the area of each block was

147 300 m^2. Banana cultivar *Musa acuminate AAA Cavendish cv*. Brazil and the pineapple

148 cultivar Golden pineapple were used in the field experiment. Three treatments were

149 assigned: (1) banana continuously cropped for two years with common organic

150 fertilizer applied (BOF); (2) banana planted after an eighteen-month pineapple

151 rotation with common organic fertilizer applied in the banana season (POF); and (3)

152 banana planted after an eighteen-month pineapple rotation treatment with bio-organic

153 fertilizer applied (PBIO). In the rotation system, pineapple and banana were planted at

154 densities of 45000 and 2,400 seedlings ha^{-1}, respectively. All organic fertilizer was

155 applied to the soil at once as base fertilizer before banana planting; other measures

156 were consistent with common banana production.

157 **2.2 Banana Fusarium wilt disease incidence statistics**

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

159 wilt symptoms of banana Fusarium wilt disease. Disease incidence was calculated

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

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

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

163 Fusarium wilt disease incidence.

164 **2.3 Soil sample collection and DNA extraction**

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

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

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

168 20 cm soil column were picked out using soil borer in each sampling site. All the 5

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

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

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

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

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

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

175 for PCR amplification.

176 **2.4 Polymerase chain reaction amplification and Illumina Miseq sequencing**

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

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

187 **2.5 Bioinformatic analysis**

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

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

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

202   principal coordinate analysis (PCoA) was set up based on the unweighted UniFrac

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

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

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

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

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

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

209   **2.6 Network analyses**

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

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

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

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

214   to visualize the network.

215   **2.7 Statistical analysis**

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

217   20.0 and R software. Pearson correlations among disease incidence, different Phylum

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

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

220   **3 Results**

221   **3.1 Disease incidence and relative abundance of *Fusarium***

222   Pineapple rotation and biofertilizer application effectively reduced Fusarium wilt

223   disease incidence and the relative abundance of *Fusarium* in the next season's banana

224   plantation (**Fig. 1A and B**). The incidences of banana Fusarium wilt disease in the

225 POF and PBIO treatments were 33.3% and 12.3%, respectively, which were

226 significantly lower than those in the BOF treatment, which reached up to 66.8%. The

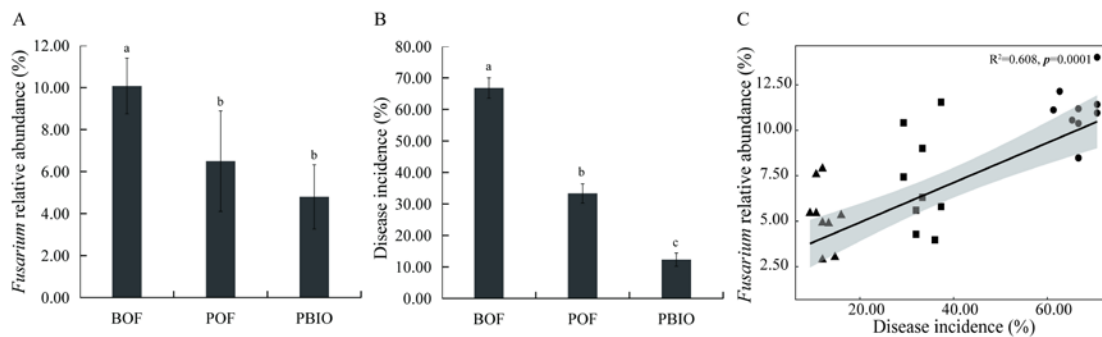
227 treatment, PBIO with rotation and biofertilizer application, showed the lowest disease

228 incidence with a 63.1% decrease compared with POF (**Fig. 1B and Table S1**). The

229 relative abundance of *Fusarium* showed the same tendency as disease incidence, and

230 disease incidence was significantly correlated with the relative abundance of

231 *Fusarium*, as revealed by MiSeq sequencing data (**Fig. 1C**).

232

233

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

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

236 abundance **(C).** BOF=banana continuously cropped with OF applied; POF= banana-pineapple

237 rotation with OF applied in the banana season; and PBIO=banana-pineapple rotation with

238 BIO applied in the banana season. Bars above the histogram represent standard errors and

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

240 analysis and multiple comparison results.

241 **3.2 General analyses of the high-throughput sequencing data**

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

243 and based on 97% similarity, a total of 8,346 16S and 5,647 ITS Operational

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

245 Bacteroidetes, Firmicutes, Proteobacteria and Verrucomicrobia were the most

246 abundant phyla, with relative abundances all greater than 1%. For fungi, Ascomycota,

followed by Basidiomycota, Chytridiomycota, Zycomycota, and Glomeromycota were the most abundant phyla (**Fig. 2**). ANOVA showed that Chlamydiae, Cyanbacteria/chloroplast, Gemmatimonadetas, Nitrospirae, Planctmycetes, and Verrucomicrobia abundances were significantly higher in the PBIO and POF treatment samples than in the BOF treatments, and the relative abundance of Ascomycota was lower in the PBIO treatment (Duncan test, $p < 0.05$).

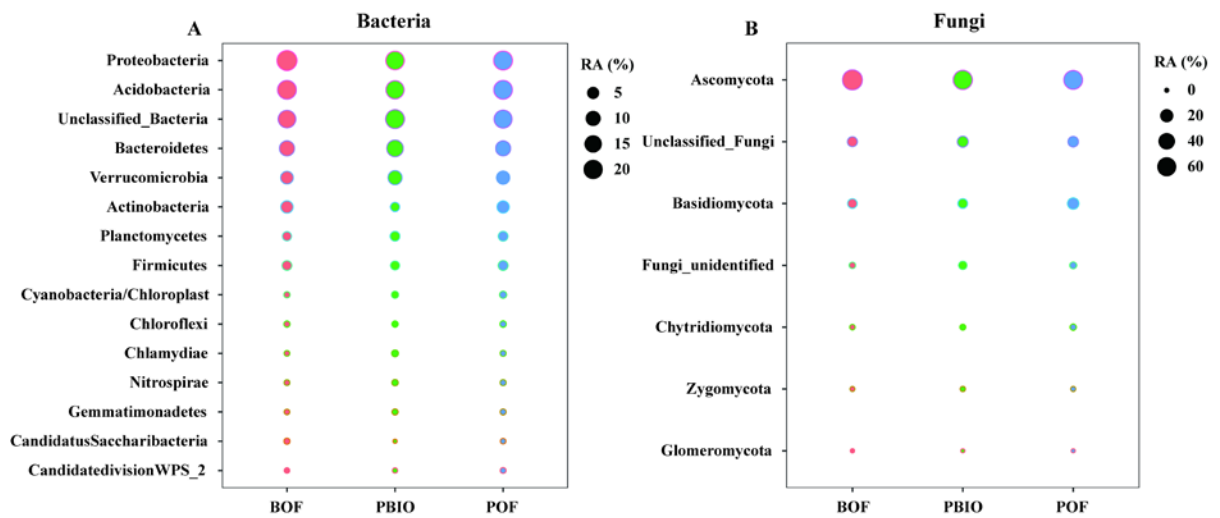



Figure 2. Bubble chart of bacterial (A) and fungal (B) phyla in BOF, POF and PBIO treatments. BOF=banana continuously cropped with OF applied; POF= banana-pineapple rotation with OF applied in the banana season; and PBIO=banana-pineapple rotation with BIO applied in the banana season; Values represent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

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

Rarefaction analyses, Chao1 and Faith's PD were performed to characterize α-diversity. Rarefaction analyses showed that the number of OTUs tended to smooth at 14,900 selected bacterial sequences and 34,943 fungal sequences. Compared with the

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

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

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

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

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

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

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

273 continuously cropped with OF applied; POF= banana-pineapple rotation with OF applied in

274 the banana season; PBIO=banana-pineapple rotation with BIO applied in the banana season;

275 Values represent the average index of nine replicates. Means followed by different letters for

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

	Treatment	Numbers of Otus	Chao1	Faith's PD
Bacteria	BOF	2606 ± 71 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

277

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

279 unweighted distance matrix to analyze differences in community composition in the

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

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

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

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

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

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

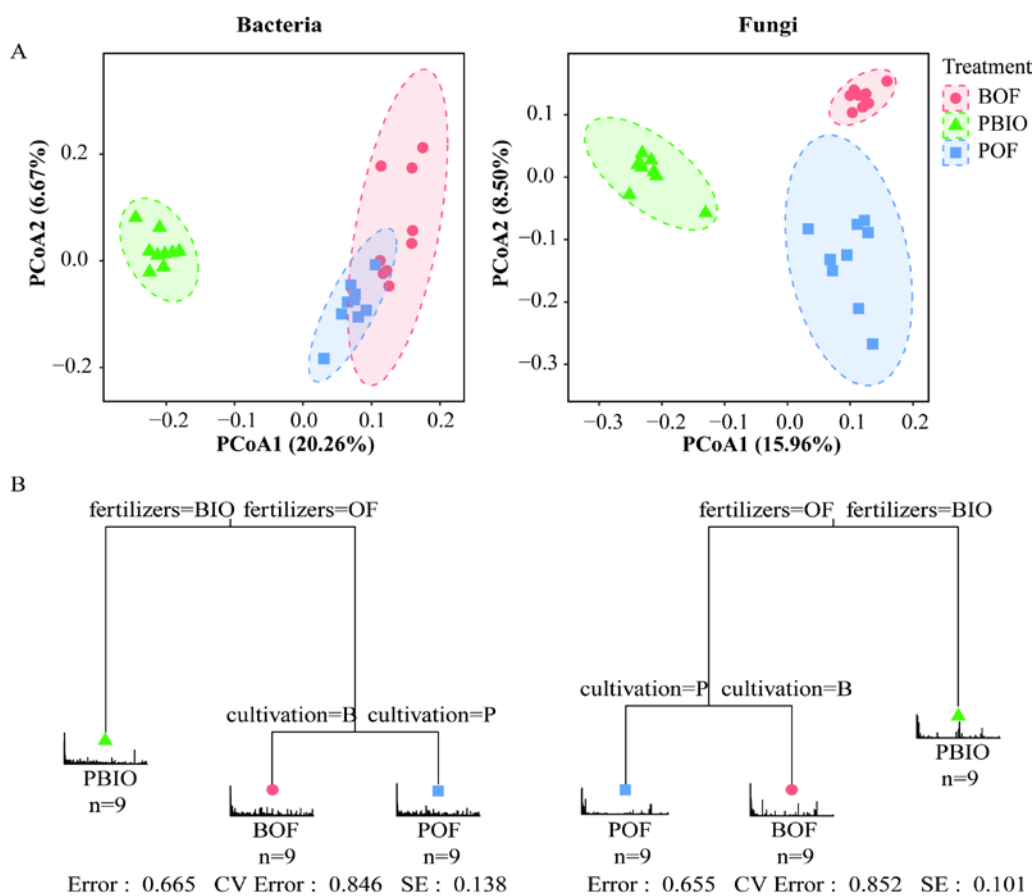
286   second component (**Fig. 3A**).

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

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

289   and that cultivation was secondarily important. Driven by fertilization, the PBIO

290   treatment was separated from the BOF and POF treatments, and then the BOF and

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

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

294   **community structures in different treatments.** BOF=banana continuously cropped with OF

295   applied; POF= banana-pineapple rotation with OF applied in the banana season;

296   PBIO=banana-pineapple rotation with BIO applied in the banana season. **(B)** Multiple

297   regression tree (MRT) analysis for the bacterial and fungal communities showed the variables

298   of fertilization and cultivation in each branch.

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

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

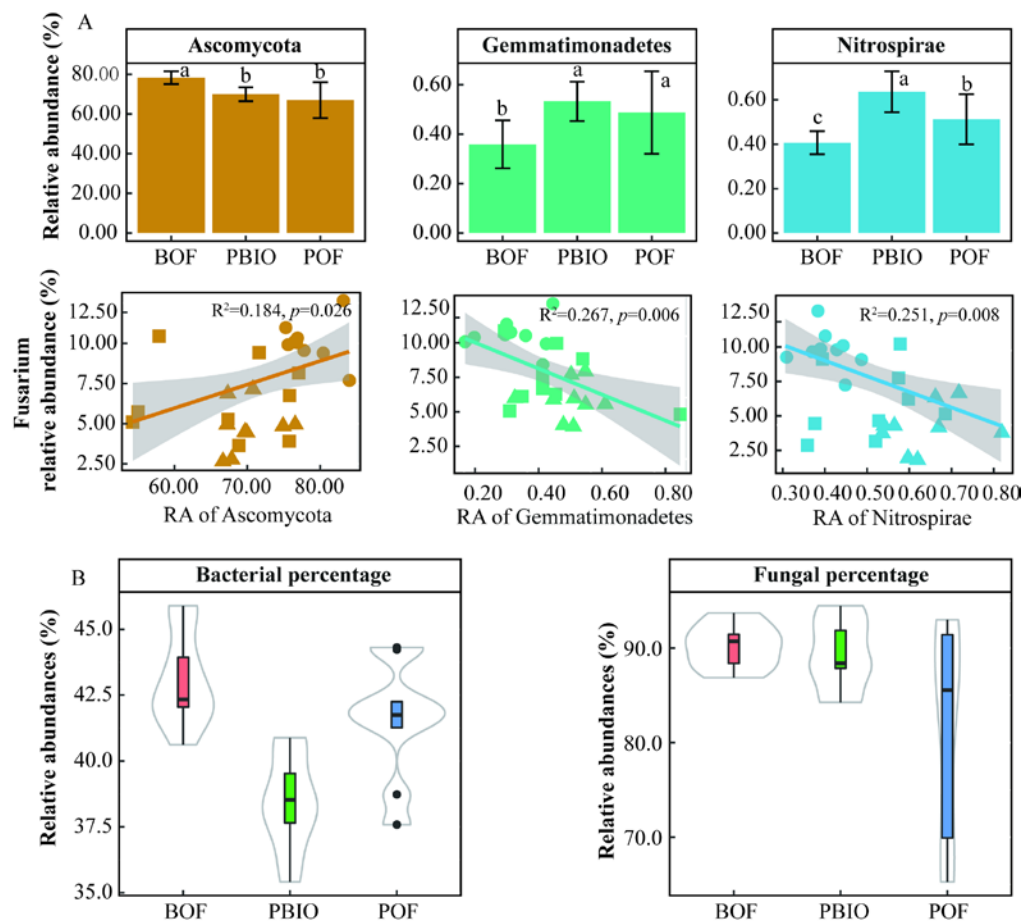



Figure 4. Relative abundance of key phyla and linear regression relationship between key phyla and disease incidence (A). Percentage of FOC related bacterial and fungal phyla in all treatments (B). Different letters above the bars indicate a significant difference at the 0.05 probability level according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

312 **3.5 Key topological properties of the networks**

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

314 those OTUs with a relative abundance greater than 0.1% were selected from each

315 treatment. A total of 301 OTUs were selected from the BOF treatment (122 bacterial

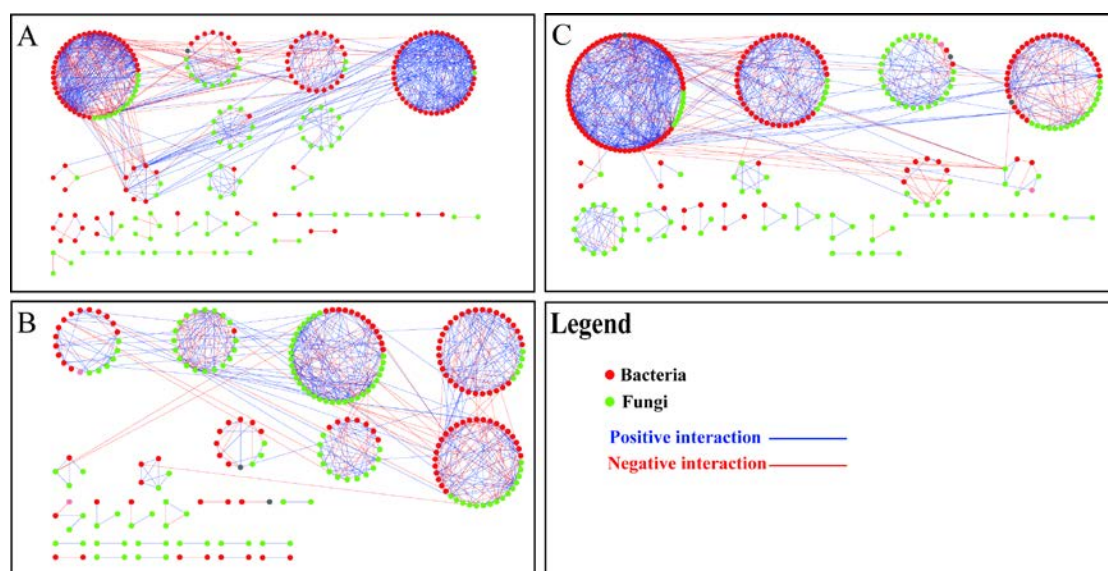
316 and 179 fungal), 323 OTUs were selected from the PBIO treatment (152 bacterial and

317 171 fungal), and 324 OTUs were selected from the POF treatment (140 bacterial and

318 184 fungal). Random matrix theory was used to build the networks. As shown in **Fig.**

319 **5**, each node represents an OTU, each link shows a significant correlation between

320 two OTUs, red and green represent bacterial and fungal OTUs, respectively, and blue

321 and red represent positive and negative correlations, respectively.

322

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 applied; POF= banana-pineapple

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

326 applied in the banana season; Red nodes indicate bacteria; Green nodes indicate fungi; red

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

328 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. F/B represents ratio of fungal to bacterial

333 nodes. The F/B ratios 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 sample, followed by the POF and BOF treatments.

336   The structure index network from the 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 applied; POF= banana-pineapple rotation with OF applied in the banana season;

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

342 connectivity; Avg CC=average clustering coefficient; and 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

343

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

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

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

347 were divided into four categories (**Fig. 6**). Three nodes were categorized as generalists

348 (module hubs) with intense connectivity to many nodes in the same modules, two

349 from the PBIO network and one from the POF network. However, no module hubs

350 were found in the BOF network. Fourteen nodes were categorized as connectors

351 (generalists) with high connectivity to several modules, eight from the BOF network

352 and six from the PBIO network. Interestingly, module hubs (generalists) were only

353 found in the pineapple-banana treatment (PBIO and POF), and connectors (generalists)

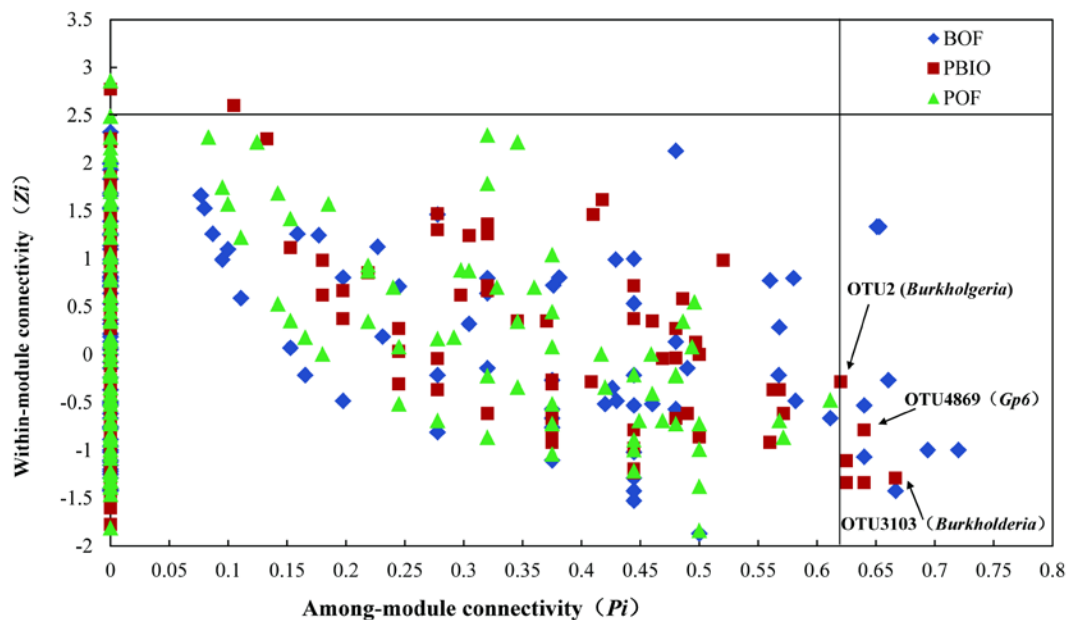
354 and module hubs (generalists) were found at the same time only in the pineapple-

355 banana with the bio-organic fertilizer applied treatment (PBIO). Annotation

356 information from all generalists showed that bacterial OTU2 and OTU3013 belonging

357 to *Burkholderia* were generalists in the PBIO network, but were absent in the POF

358 and BOF networks. Additionally, another generalist OTU4869, from the PBIO

359 network was identified as *Gp6* in Acidobacteria.

360

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

362 Each symbol represents an OTU in different treatment. BOF=banana continuously cropped

363 with OF applied; POF= banana-pineapple rotation with OF applied in the banana season;

364 PBIO=banana-pineapple rotation with BIO applied in the banana season; The threshold

365 values of *Zi* and *Pi* for categorizing OTUs were 2.5 and 0.62, respectively, as proposed by

366 Guimera and Amaral (2005) and simplified by Olesen et al. (2007).

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

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

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

	df	F	*P*	Relative Importance
Bac-PCoA1	**1**	**304.09**	**<0.0001**	**19.32%**
Fun-PCoA1	1	1.11	0.31	16.32%
Bac-Chao1	1	4.10	0.062	9.60%
Fun-Chao1	1	1.11	0.309	4.19%
Bac-Faith's PD	1	1.59	0.227	6.80%
Fun-Faith's PD	1	1.64	0.221	6.05%
Ascomycota	1	2.11	0.168	1.93%
Fusarium Relative abundance	1	1.01	0.332	8.23%
Nitrospirae	1	0.88	0.363	7.29%
Gemmatimonadetes	1	0.04	0.852	2.61%
Burkholderia	1	0.76	0.399	10.17%
Bacillus	1	0.33	0.574	3.29%
Residuals	14			

Model summary: R^2=0.9417, AIC =123.26, p < 0.0001

Total response variance: 95.79%

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

382   In addition, based on linear regression analyses between disease incidence and

383   selected microbial indicators, we found that bacterial structure (F = 304.09, p <

384   0.0001, relative importance = 19.32%) had a significant relationship with disease

385   incidence.

386   **4 Discussion**

387   In our previous research, the effectiveness of pineapple-banana rotation and bio-

388   organic fertilizer application were proven in the control of banana Fusarium wilt

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

390   important indicator for exploring the mechanisms behind these two control measures.

391   In this study, disease incidence and soil microbial community characteristics during

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

393   impact of rotation and bio-organic fertilizer application.

394   Our previous results indicated that the pineapple-banana rotation treatments

395   significantly reduced Fusarium wilt disease incidence when compared with banana

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

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

398   fumigation with lime and ammonium bicarbonate was highly effective in controlling

399   banana Fusarium wilt disease. Thus, although many measures can slow down the

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

401   use of more than one measure (Pda et al., 2017). Therefore, in the current study, we

402   explored the combined use effect of pineapple-banana rotation and bio-organic

403   fertilizer application to provide a promising strategy to manage banana Fusarium wilt

404   disease. The results were consistent with previous reports.

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

406 biofertilizer treatments. Previous studies have shown a high positive correlation

407 between disease suppression and a high diversity of bacteria with a concurrent low

408 diversity of fungi (Bonanomi et al., 2010; Fu et al., 2017). However, inconsistent with

409 these results, the pineapple-banana rotation and biofertilizer treatment (PBIO)

410 harbored a significantly higher fungal richness and diversity than the other two

411 treatments (BOF and POF). This agrees with two other previous studies that indicated

412 the importance of fungal diversity in the suppressive capacity of vanilla soils and

413 potato cropping systems (Xiong et al., 2017). Many previous studies have shown that

414 a decrease in soil pH is an important factor leading to soil-borne diseases. Microbial

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

416 et al., 2013). We observed that soil pH increased in the rotation and bio-organic

417 fertilizer treatments (**Table S2**); therefore, the high bacterial and fungal diversity

418 observed in our rotation and bio-organic fertilizer system may have been due to the

419 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 bio-organic fertilizer application (Sun et al., 2015) altered the soil

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

425 revealed fertilization effects on microbial community composition, indicating that

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

427 in determining microbial community composition. These results were similar to

428 previous results where bio-organic fertilizer application was the greatest factor in

429 determining microbial community composition rather than temporal variability (Fu et

430 al., 2017). This is also a powerful illustration of the necessity of bio-organic fertilizer

431 application in pineapple-banana rotation.

432   Phylum-level results show that rotation and biofertilizer application decreased

433 the relative abundance of Ascomycota, and increased the relative abundance of

434 Chlamydiae, Gemmatimondetes, Nitrospire, Planctomycetes, and Verrucomicrobia,

435 which were all associated with disease suppression in previous reports (Trivedi et al.,

436 2017; Shen et al., 2018). Our fungal results are consistent with previous observations

437 of low Ascomycota phylum abundance in suppressed soil, which is logical because

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

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

440 in Ascomycetes was considered an important factor in FOC decrease during the

441 pineapple season (Wang et al., 2015). The bacterial results are partly consistent with

442 previous observations that rotations with wild rocket and Indian mustard increased the

443 *Nitrospira* and Gemmatimonadetes contents (Jin et al., 2019). It is worth noting that

444 our BIO treatment was secondary fermentation with *Bacillus* added, while the

445 *Bacillus* genus was not enriched in the BIO treatment soil. Moreover, microbial

446 structure appeared to be the most constrained factor with disease incidence in linear

447 models between microbial indicators and the incidence of banana Fusarium wilt

448 disease. Xiong et al (2017) suggested that microbial species introduced by

449 biofertilizer application induced wilt suppression by microbiome transformation

450 rather than pathogen suppression directly. Alteration of the soil microbiome may

451 cause a greater response than the added *Bacillus* in the PBIO treatment sample in our

452 case.

453   We previously confirmed that pineapple-banana rotation reduced the amount of

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

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

456 percentage of FOC-correlated fungi genera was observed in all treatments. Even

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

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

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

460 soil factor 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 the fungal community in both the pineapple

464 and banana seasons further reinforced the importance of fungal community changes in

465 our case.

466   Several researchers have used microbial molecular ecological networks to study

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

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

469 molecular ecological networks revealing distinct differences between the microbial

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

471 were selected 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 were only a part of the entire system, there is no doubt that these OTUs were 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 have led to changes in soil

476 function. PBIO, POF, and BOF soils harbored modules with modularity values of

477 0.718, 0.642, and 0.616, respectively, in this study. Modularity represents how well

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

479 possessed high modularity, had more connections between nodes in the same modules,

480 followed by the POF and BOF networks. The altered networks compared with POF

481 and BOF networks may have partially contributed latent attributes to higher disease

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

483   no module hubs (generalists) were present in the BOF network, whereas all three

484   module hubs were found in the pineapple-banana rotation network, as indicated by the

485   *Zi-Pi* relationship. In all three networks, connectors (generalists) and module hubs

486   (generalists) were found at the same time only in the PBIO treatment. Generalists

487   typically only occupy a small fraction of a community; however, the presence of those

488   generalists is very important (Zhou et al., 2011; Jens et al., 2011). These nodes could

489   have enhanced connecters within or among modules. If the network is poorly

490   connected or not connected at all, the community is predicted to be disordered, and

491   fluxes of energy, material, and information would not be efficient (Lu et al., 2013).

492   Therefore, in our case, these generalists found in the PBIO treatment suggest that the

493   microbial community structure was more orderly and powerful than in the other two

494   treatments.

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

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

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

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

499   antagonistic *Burkholderia* were found during the pineapple season in our previous

500   report (Wang et al., 2015). In addition, our linear model analysis shows that in

501   addition to bacterial and fungal structure, *Burkholderia* relative abundance

502   constrained disease incidence with a high relative importance factor of 10.17%.

503   *Burkholderia* is a versatile organism due to its powerful ability to occupy ecological

504   niches and a variety of functions, including biological control and plant growth

505   promotion, in agriculture (Coenye and Vandamme, 2003). This suggests that even

506   though the relative abundance of *Burkholderia* in the PBIO treatment was not that

507   high, the change in network structure in the rotation and bio-organic fertilizer

508   treatments may have been attributed to the general wilt suppression activity, and that

509   change may have specifically been due to the special functions of *Burkholderia*.

510   Additionally, one generalist in the PBIO treatment sample was identified as *Gp6* in

511   Acidobacteria. Although no Acidobacteria antimicrobial activities have previously

512   been recorded, several studies have demonstrated that Acidobacteria is greatly

513   affected by soil pH and that *Gp6* is positively correlated with soil pH (Bartram et al.,

514   2014; Jones et al., 2009). Therefore, the special function of *Gp6* in the PBIO network

515   probably resulted from an increase in soil pH.

516   **5 Conclusions**

517   An expansion of previous work revealed that pineapple-banana rotation combined

518   with bio-organic fertilizer application during the banana season was effective in

519   reducing *Fusarium* spp. abundance and banana Fusarium wilt. Several different

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

521   were changed by rotation and bio-organic fertilizer application. Bio-organic fertilizer

522   inhibited Fusarium wilt disease by changing the soil microbial structure, rather than

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

524   *Burkholderia* functions in the network were likely the most responsible factors for

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

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

527   Fusarium wilt disease.

528   **Data availability**

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

530   Sequence Read Archive database (SRP234066).

531   **Author contributions**

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

Competing interests

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

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).

References

Alabouvette, C., Olivain, C., Migheli, Q., and Steinberg, C.: Microbiological control of soil-borne phytopathogenic fungi with special emphasis on wilt-inducing Fusarium oxysporum, New. Phytol., 184, 529-544, https://doi.org/10.1111/j.1469-8137.2009.03014.x, 2009.

Bartram, A. K., Jiang, X., Lynch, M. D. J., Masella, A. P., Nicol, G. W., Jonathan, D., and Neufeld, J. D.: Exploring links between pH and bacterial community composition in soils from the Craibstone Experimental Farm, FEMS Microbiol. Ecol., 403, https://doi.org/10.1111/1574-6941.12231, 2014.

Bonanomi, G., Antignani, V., Capodilupo, M., and Scala, F.: Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases, Soil Biol. Biochem., 42, 136-144, https://doi.org/10.1016/j.soilbio.2009.10.012, 2010.

Bullock, D. G.: Crop rotation, Crit Rev Plant Sci., 11, 309-326, https://doi.org/10.1080/07352689209382349, 1992.

561 Butler, D.: Fungus threatens top banana, Nature., 504, 195-196,
562   https://doi.org/10.1038/504195a, 2013.

563 Cai, F., Pang, G., Li, R. X., Li, R., Gu, X. L., Shen, Q. R., and Chen, W.: Bioorganic
564   fertilizer maintains a more stable soil microbiome than chemical fertilizer for
565   monocropping, Biol.Fertil. Soils., 53, 861-872, https://doi.org/10.1007/s00374-
566   017-1216-y, 2017.

567 Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello,
568   E. K., Fierer, N., Peña, A., Goodrich, J. K., and Gordon, J. I.: QIIME allows
569   analysis of high-throughput community sequencing data, Nat. Methods., 7, 335–
570   336, https://doi.org/10.1038/nmeth.f.303, 2010.

571 Cha, J. Y., Han, S., Hong, H. J., Cho, H., and Kwak, Y. S.: Microbial and biochemical
572   basis of a Fusarium wilt-suppressive soil, ISME J., 10, 119-129,
573   https://doi.org/10.1038/ismej.2015.95, 2016.

574 Chaparro, J. M., Sheflin, A. M., Manter, D. K., and Vivanco, J. M.: Manipulating the
575   soil microbiome to increase soil health and plant fertility, Biol.Fertil. Soils., 48,
576   489-499, https://doi.org/10.1007/s00374-012-0691-4, 2012.

577 Christen, O. and Sieling, K.: Effect of Different Preceding Crops and Crop Rotations
578   on Yield of Winter Oil-seed Rape (*Brassica napus L.*), J Agron Crop Sci., 174,
579   265-271, https://doi.org/10.1111/j.1439-037X.1995.tb01112.x, 2010.

580 Claesson, M. J., O'Sullivan, O., Wang, Q., Nikkila¨, J., and Marchesi, J. R.:
581   Comparative Analysis of Pyrosequencing and a Phylogenetic Microarray for
582   Exploring Microbial Community Structures in the Human Distal Intestine, PLoS
583   One., 4, e6669-, https://doi.org/10.1371/journal.pone.0006669, 2009.

584 Coenye, T. and Vandamme, P.: Diversity and significance of Burkholderia species
585   occupying diverse ecological niches, Environ. Microbiol., 5, 719-729,
586   https://doi.org/10.1046/j.1462-2920.2003.00471.x, 2003.

587 Coyte, K. Z., Schluter, J., and Foster, K. R.: The ecology of the microbiome:
588   Networks, competition, and stability, Science., 350, 663-666,
589   https://doi.org/10.1126/science.aad2602, 2015.

590 Deng, Y., Jiang, Y., Yang, Y., He, Z., Luo, F., and Zhou, J.: Molecular ecological
591   network analyses, BMC Bioinformatics., 13, 113, https://doi.org/10.1186/1471-
592   2105-13-113, 2012.

593 Duniway, J. M.: Status of Chemical Alternatives to Methyl Bromide for Pre-Plant
594   Fumigation of Soil, Phytopathology., 92, 1337-1343,
595   https://doi.org/10.1094/PHYTO.2002.92.12.1337, 2003.

596 Edgar, R. C.: UPARSE: highly accurate OTU sequences from microbial amplicon
597   reads, Nat. Methods., 10, 996-998, https://doi.org/10.1038/nmeth.2604, 2013.

598 Faith, D. P.: Conservation evaluation and phylogenetic diversity, Biol. Conserv., 61,
599   1-10, https://doi.org/10.1016/0006-3207(92)91201-3, 1992.

600 Fravel, D., Olivain, C., and Alabouvette, C.: Fusarium oxysporum and its biocontrol,
601   New. Phytol., 157, 493-502, https://doi.org/10.1046/j.1469-8137.2003.00700.x,
602   2003.

603 Fu, L., Ruan, Y., Tao, C., Li, R., and Shen, Q.: Continous application of bioorganic
604   fertilizer induced resilient culturable bacteria community associated with banana
605   Fusarium wilt suppression, Sci. Rep., 6, 27731,
606   https://doi.org/10.1038/srep27731, 2016.

607 Fu, L., Penton, C. R., Ruan, Y., Shen, Z., Xue, C., Li, R., and Shen, Q.: Inducing the
608   rhizosphere microbiome by biofertilizer application to suppress banana Fusarium
609   wilt disease, Soil Biol. Biochem., 104, 39-48,
610   https://doi.org/10.1016/j.soilbio.2016.10.008, 2017.

611 Gerbore, J., Benhamou, N., Vallance, J., Floch, G., Grizard, D., Regnault-Roger, C.,
612   and Rey, P.: Biological control of plant pathogens: advantages and limitations
613   seen through the case study of Pythium oligandrum, Environ. Sci. Pollu.t R., 21,
614   4847-4860, https://doi.org/10.1007/s11356-013-1807-6, 2014.

615 Hartmann, M., Frey, B., Mayer, J., Mäder, P., and Widmer, F.: Distinct soil microbial
616   diversity under long-term organic and conventional farming, ISME J., 9, 1177-
617   1194, https://doi.org/10.1038/ismej.2014.210, 2015.

618 Helena, C., Lurdes, B., Susana, R.-E., and Helena, F.: Trends in plant and soil
619   microbial diversity associated with Mediterranean extensive cereal-fallow
620   rotation agro-ecosystems, Agric., Ecosyst. Environ., 217, 33-40,
621   https://doi.org/10.1016/j.agee.2015.10.027., 2016.

622 Jens, M. O., Jordi, B., Yoko L, D., and Pedro, J.: The modularity of pollination
623   networks, Proc. Natl. Acad. Sci. USA., 104, 19891-19896,
624   https://doi.org/10.1073/pnas.0706375104, 2011.

625 Jin, X., Wang, J., Li, D., Wu, F., and Zhou, X.: Rotations with Indian Mustard and
626   Wild Rocket Suppressed Cucumber Fusarium Wilt Disease and Changed

Rhizosphere Bacterial Communities, Microorganisms., 7, 57, https://doi.org/10.3390/microorganisms7020057, 2019.

Jones, R. T., Robeson, M. S., Lauber, C. L., Hamady, M., Knight, R., and Fierer, N.: A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses, ISME J., 3, 442-453, https://doi.org/10.1038/ismej.2008.127, 2009.

Krupinsky, J. M., Bailey, K. L., Mcmullen, M. P., Gossen, B. D., and Turkington, T. K.: Managing Plant Disease Risk in Diversified Cropping Systems, Agron J., 94, 198-209, https://doi.org/10.2134/agronj2002.1980, 2002.

Le, C. R., Simon, T. E., Patrick, D., Maxime, H., Melen, L., Sylvain, P., and Sabrina, S.: Reducing the Use of Pesticides with Site-Specific Application: The Chemical Control of Rhizoctonia solani as a Case of Study for the Management of Soil-Borne Diseases, PLoS One., 11, e0163221-, https://doi.org/10.1371/journal.pone.0163221, 2016.

Liu, J., Sui, Y., Yu, Z., Shi, Y., Chu, H., Jin, J., Liu, X., and Wang, G.: High throughput sequencing analysis of biogeographical distribution of bacterial communities in the black soils of northeast China, Soil Biol. Biochem., 70, 113-122, https://doi.org/10.1016/j.soilbio.2013.12.014, 2014.

Liu, L., Kong, J., Cui, H., Zhang, J., Wang, F., Cai, Z., and Huang, X.: Relationships of decomposability and C/N ratio in different types of organic matter with suppression of Fusarium oxysporum and microbial communities during reductive soil disinfestation, Biol .Control., 101, 103-113, https://doi.org/10.1016/j.biocontrol.2016.06.011, 2016.

Lozupone, C., Hamady, M., and Knight, R.: UniFrac–An online tool for comparing microbial community diversity in a phylogenetic context, Nat. New Biol., 241, 184-186, https://doi.org/10.1186/1471-2105-7-371, 2005.

Lu, L., Yin, S., Liu, X., Zhang, W., Gu, T., Shen, Q., and Qiu, H.: Fungal networks in yield-invigorating and -debilitating soils induced by prolonged potato monoculture, Soil Biol. Biochem., 65, 186-194, https://doi.org/10.1016/j.soilbio.2013.05.025, 2013.

Lupwayi, N. Z., Rice, W. A., and Clayton, G. W.: Soil microbial diversity and community structure under wheat as influenced by tillage and crop rotation, Soil Biol. Biochem., 30, 1733-1741, https://doi.org/10.1016/S0038-0717(98)00025-X, 1998.

Mazzola, M. and Freilich, S.: Prospects for Biological Soilborne Disease Control: Application of Indigenous Versus Synthetic Microbiomes, Phytopathology., 107, 256, https://doi.org/10.1094/PHYTO-09-16-0330-RVW, 2017.

Mendes, L. W., Kuramae, E. E., Navarrete, A. A., Veen, J. V., and Tsai, S. M.: Taxonomical and functional microbial community selection in soybean rhizosphere, ISME J., 8, 1577-1587, https://doi.org/10.1038/ismej.2014.17, 2014.

Mona, N., Högberg., Stephanie, A., Yarwood., and David, D., Myrold.: Fungal but not bacterial soil communities recover after termination of decadal nitrogen additions to boreal forest, Soil Biol. Biochem., 2014,72, 35-43, https://doi.org/10.1016/j.soilbio.2014.01.014, 2014.

Nel, B., Steinberg, C., Labuschagne, N., and Viljoen, A.: Evaluation of fungicides and sterilants for potential application in the management of Fusarium wilt of banana, Crop Prot., 26, 697-705, https://doi.org/10.1016/j.cropro.2006.06.008, 2007.

Pda, B., Scf, A., Olp, B., Ic, C., D , S., Jd, E., and Mh, A.: Disease suppressiveness to Fusarium wilt of banana in an agroforestry system: Influence of soil characteristics and plant community, Agric., Ecosyst. Environ., 239, 173-181, https://doi.org/10.1186/1471-2105-7-371, 2017.

Ploetz, R. C.: Fusarium Wilt of Banana, Phytopathology., 105, 1512, 2015.

Qiu, MH, Zhang, RF, Xue, SS, SQ, Shen, and QR: Application of bio-organic fertilizer can control Fusarium wilt of cucumber plants by regulating microbial community of rhizosphere soil, Biol.Fertil. Soils., 48, 807-816, https://doi.org/10.1007/s00374-012-0675-4, 2012.

Robert, P., Larkin, J., M., and Halloran.: Management Effects of Disease-Suppressive Rotation Crops on Potato Yield and Soilborne Disease and Their Economic Implications in Potato Production, Am J Potato Res., 91, 429-439, https://doi.org/10.1007/s12230-014-9366-z, 2014.

Schloss, P., Westcott, S., Ryabin, T., Hall, J., Hartmann, M., Hollister, E., Lesniewski, R., Oakley, B., Parks, D., Robinson, C., Sahl, J., Stres, B., Thallinger, G., Van Horn, D., and Weber, C.: Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities, Appl. Environ. Microbiol., 75, 7537-7541, https://doi.org/10.1128/AEM.01541-09, 2009.

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., and Chen, W.: Nuclear ribosomal internal transcribed spacer (ITS) region as a

695   universal DNA barcode marker for Fungi, P Natl Acad Sci USA., 109, 6241-
696   6246, https://doi.org/10.1073/pnas.1117018109, 2012.

697   Shen, C., Xiong, J., Zhang, H., Feng, Y., Lin, X., Li, X., Liang, W., and Chu, H.: Soil
698   pH drives the spatial distribution of bacterial communities along elevation on
699   Changbai   Mountain,   Soil   Biol.   Biochem.,   57,   204-211,
700   https://doi.org/10.1016/j.soilbio.2012.07.013, 2013.

701   Shen, Z., Ruan, Y., Xue, C., Zhang, J., and Li, R.: Rhizosphere microbial community
702   manipulated by 2 years of consecutive biofertilizer application associated with
703   banana Fusarium wilt disease suppression, Biol.Fertil. Soils., 51, 553–562,
704   https://doi.org/10.1007/s00374-015-1002-7, 2015.

705   Shen, Z., Xue, C., Taylor, P., Ou, Y., Wang, B., Zhao, Y., Ruan, Y., Li, R., and Shen,
706   Q.: Soil pre-fumigation could effectively improve the disease suppressiveness of
707   biofertilizer to banana Fusarium wilt disease by reshaping the soil microbiome,
708   Biol.Fertil. Soils., 54, 793–806, https://doi.org/10.1007/s00374-018-1303-8,
709   2018.

710   Sun, R., Zhang, X. X., Guo, X., Wang, D., and Chu, H.: Bacterial diversity in soils
711   subjected to long-term chemical fertilization can be more stably maintained with
712   the addition of livestock manure than wheat straw, Soil Biol. Biochem., 88, 9-18,
713   https://doi.org/10.1016/j.soilbio.2015.05.007, 2015.

714   Trivedi, P., Delgado-Baquerizo, M., Trivedi, C., Hamonts, K., Anderson, I. C., and
715   Singh, B. K.: Keystone microbial taxa regulate the invasion of a fungal pathogen
716   in   agro-ecosystems,   Soil   Biol.   Biochem.,   111,   10-14,
717   https://doi.org/10.1016/j.soilbio.2017.03.013, 2017.

718   Wang, B., Li, R., Ruan, Y., Ou, Y., and Zhao, Y.: Pineapple–banana rotation reduced
719   the amount of Fusarium oxysporum more than maize–banana rotation mainly
720   through modulating fungal communities, Soil Biol. Biochem., 86, 77-86,
721   https://doi.org/10.1016/j.soilbio.2015.02.021, 2015.

722   Wang, B., Yuan, J., Zhang, J., Shen, Z., Zhang, M., Li, R., Ruan, Y., and Shen, Q.:
723   Effects of novel bioorganic fertilizer produced by Bacillus amyloliquefaciens
724   W19 on antagonism of Fusarium wilt of banana, Biol.Fertil. Soils., 49, 435-446,
725   https://doi.org/10.1007/s00374-012-0739-5, 2013.

726   Wang, Q., Garrity, G. M., Tiedje, J. M., and Cole, J. R.: Nave Bayesian Classifier for
727   Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy, Appl.
728   Environ. Microbiol., 73, 5261, https://doi.org/10.1128/AEM.00062-07, 2007.

Xiong, W., Li, R., Ren, Y., Liu, C., Zhao, Q., Wu, H., Jousset, A., and Shen, Q.: Distinct roles for soil fungal and bacterial communities associated with the suppression of vanilla Fusarium wilt disease, Soil Biol. Biochem., 107, 198-207, https://doi.org/10.1016/j.soilbio.2017.01.010, 2017.

Xiong, W., Zhao, Q., Xue, C., Xun, W., Zhao, J., Wu, H., Rong, L., and Shen, Q.: Comparison of Fungal Community in Black Pepper-Vanilla and Vanilla Monoculture Systems Associated with Vanilla Fusarium Wilt Disease, Front. Microbiol., 7, 117, https://doi.org/10.3389/fmicb.2016.00117, 2016.

Yin, W., Jie, X., Shen, J., Luo, Y., Scheu, S., and Xin, K.: Tillage, residue burning and crop rotation alter soil fungal community and water-stable aggregation in arable fields, Soil Till Res., 107, 71-79, https://doi.org/10.1016/j.still.2010.02.008, 2010.

Zhang, F., Zhen, Z., Yang, X., Wei, R., and Shen, Q.: Trichoderma harzianum T-E5 significantly affects cucumber root exudates and fungal community in the cucumber rhizosphere, Appl. Soil Ecol., 72, 41-48, https://doi.org/10.1016/j.apsoil.2013.05.016, 2013a.

Zhang, H., Mallik, A., and Zeng, R. S.: Control of Panama Disease of Banana by Rotating and Intercropping with Chinese Chive (Allium Tuberosum Rottler): Role of Plant Volatiles, J. Chem. Ecol., 39, 243-252, https://doi.org/10.1007/s10886-013-0243-x, 2013b.

Zhou, J., Deng, Y., Luo, F., He, Z., and Yang, Y.: Phylogenetic Molecular Ecological Network of Soil Microbial Communities in Response to Elevated CO_2, mBio., 2, : e00122-00111., http://mbio.asm.org/content/2/4/e00122-11.short, 2011.