

# Manipulating the soil microbial community during a community recovery process with plant beneficial species for the suppression of Fusarium wilt of watermelon

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## Original article

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1           **Manipulating the soil microbial community during a**  
2 **community recovery process with plant beneficial species for**  
3 **the suppression of Fusarium wilt of watermelon**

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13 **Keywords:** Watermelon Fusarium wilt disease; Soil microbial resistance and  
14 resilience; Dazomet fumigation; Biological organic fertilizer; Soil microbial  
15 assembly.

16 **ABSTARCT**

17 Fusarium wilt is a devastating disease which impacts watermelon production. Soil  
18 fumigation using dazomet followed by biological organic fertilizer was applied to  
19 suppress the Fusarium wilt disease. We propose that fumigation suppresses the soil  
20 indigenous community, especially the soil-borne disease pathogens, while the  
21 utilization of bio-organic fertilizer facilitates the recovery of the soil microbiome to a  
22 beneficial, suppressive state through the introduction of plant growth-promoting

23 microorganisms. Greenhouse experiments showed that applied biological organic  
24 fertilizer after dazomet fumigation effectively restrained the disease incidence with a 93.6%  
25 disease control. Fumigation strongly decreased soil microbial diversity and altered  
26 relative taxa abundances, suggesting the possibility of niche release by the resident soil  
27 microbial community. Fumigation followed by bio-fertilizer transformed the soil  
28 microbial community composition and resulted in higher relative abundances of  
29 beneficial microbial groups such as *Bacillus* (8.5 %) and *Trichoderma* (13.5 %),  
30 coupled with lower *Fusarium* abundance compared to other treatments. Network  
31 analysis illustrated that soil fumigation decreased interactions within the soil microbial  
32 community with less nodes and links while bio-fertilizer addition promoted node  
33 interactions. In addition, bio-fertilizer addition after fumigation resulted in the  
34 beneficial species becoming the key network connectors. Collectively, fumigation  
35 appears to release the resident soil niche resulting in lower diversity while the beneficial  
36 microbes introduced by bio-fertilizer addition colonize these niches, leading to a more  
37 complex community with fewer pathogens that suppresses *Fusarium* wilt disease  
38 incidence.

39 **KEYWORDS:** Watermelon *Fusarium* wilt disease; Soil microbial resistance and  
40 resilience; Dazomet fumigation; Biological organic fertilizer; Soil microbial assembly.

41

42 **INTRDUCTION**

43

44 Watermelon Fusarium wilt as a serious plant disease worldwide was arose from the  
45 pathogenic fungus *Fusarium oxysporum* f. sp. *niveum* (FON) (Ling et al., 2010a). which  
46 negatively restrains watermelon production worldwide (Zhou and Everts, 2004).  
47 Dazomet (tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione) is often employed to  
48 suppress serious *Fusarium* infection in watermelon (Slusarski and Pietr, 2009; Tian et  
49 al., 2014). When dazomet is applied to moist and hyperthermal soils, the fumigant  
50 decomposes into methyl isothiocyanate (MITC), which can effectively suppress fungi,  
51 nematodes, and weeds (Saeed et al., 2007). Many beneficial antergic species, for  
52 instance, *Bacillus* spp., and *Trichoderma* spp., which isolated from suppressive soil  
53 were utilized for the suppression of Fusarium wilt disease already (La Fuente et al.,  
54 2006; Qiu et al., 2012; Yuan et al., 2016). Beneficial species premixed with compost  
55 and amino acid as effective fertilizers in the suppression of many cash crops soil-borne  
56 disease have been widely reported (Luo et al., 2010; Qiu et al., 2012; Wu et al., 2014).  
57 The extra spaces and nutrients provided by bio-organic amendments for the antergic  
58 species that facilitates their colonization and pathogen antagonism (Xiong et al., 2017a).  
59 Such fertilizers increase the soil fertility and reduce the adverse impact of disease  
60 pathogens, thereby improve the plant vitality (Bailey and Lazarovits, 2003).  
61 Nevertheless, few is understood concerning the underlying mechanisms of fumigation  
62 using dazomet followed by biological organic fertilizer amendment during the process  
63 of soil-borne disease inhibition.

64 Soil microbes have a determinate place within the sustainability of soil biological  
65 activity, maintaining ecological balance for the soil ecosystem and plant production  
66 through their metabolic activities and energy exchanges. It is necessary to understand  
67 the contribution of soil microbial community resistance and resilience during the  
68 manipulation of the resident soil microflora for the disease suppression (Allison and  
69 Martiny, 2008). A outside disturbance is often caused by biotic or abiotic elements,  
70 which leads to a microbial community tiny destabilization or a ‘stress’, such as the  
71 reaction from the independent or the whole ecological system. On the basis of their  
72 length of time, disturbances are usual divided into pulses and presses, which effect the  
73 soil physicochemical properties (Rykiel, 1985). In general, fumigation can be  
74 recognized as a ‘disturbance’, which is a pulse disturbance as it is a short-term event.  
75 Dazomet fumigation may exert a toxic impact on the microbial community because of  
76 the methyl-isothiocyanate and sulphidric acid it volatilizes which negatively effects soil  
77 microorganisms, including the beneficial species (Scopa and Dumontet, 2007).  
78 Ultimately, the recovery of soil productivity is dependent on the re-establishment of the  
79 soil microbial activity and the creation of a biological environment for soil diseases  
80 control (Eo and Park, 2014). In consideration of the decisive importance of maintaining  
81 soil microbial ecosystems for long-term productivity, large numbers of vigor on  
82 realizing the reactivity (including resistance and resilience) of microbial community to  
83 extraneous disturbances has been put into effect (Wertz et al., 2007; Fujino et al., 2008).  
84 Fumigation followed by the biological organic fertilizer application amendment is able

85 to suppress soil-borne wilt disease through decreasing the number of pathogens and by  
86 modifying the resident soil microbial community diversity and/or structure.

87 Our hypothesis is that soil fumigation will overcome the inherent resistance of  
88 microbial community also suppress the resident soil community, including both  
89 pathogens and beneficial species. Beneficial microbiota introduced through bio-  
90 fertilizer application will further suppress pathogen growth while promoting crop  
91 viability through manipulating the resident microbial community during the period of  
92 community resilience. To test our hypothesis, greenhouse experiment was carried out  
93 to confirm the effectiveness of the biological organic fertilizer application after  
94 dazomet fumigation on the control of watermelon Fusarium wilt disease. The soil  
95 samples were gathered, and soil microbial composition and diversity were accessed  
96 after soil fumigation and biofertilizer application. Microbial ecological network was  
97 established with the purpose of exploring community interaction and complexity to  
98 reveal the effect of an integrated agricultural strategy on soil microflora.

99

## 100 **MATERIALS AND METHODS**

101

### 102 ***Microorganisms, organic and biological organic fertilizer preparation***

103

104 Antagonistic bacterium *Bacillus amyloliquefaciens* SQR9 and the fungal strain  
105 *Trichoderma guizhouense* NJAU4742 were separated by the Jiangsu Provincial Key  
106 Lab for Organic Solid Waste Utilization, Nanjing Agricultural University. Both strains

107 showed negative impact with the fungal pathogen *F. oxysporum* growth (Cao et al.,  
108 2011; Yuan et al., 2016). Bio-fertilizer preparation process was described previously, at  
109 a word, a certain number of antagonistic strain SQR9 and NJAU4742 were added into  
110 compost for second solid fermentation and finally reach the concentration of  $10^9$  CFU  
111  $\text{g}^{-1}$  and  $10^8$  CFU  $\text{g}^{-1}$ , respectively (Ling et al., 2010b; Zhang et al., 2008).

112

### 113 ***Field experiment***

114

115 Field experiment was conducted in Huaian (33°35' N, 119°01' E), Jiangsu Province,  
116 China from March to July 2017. Watermelon was continuously cultivated in the  
117 greenhouse for more than 4 years (8 growing seasons). The Fusarium wilt disease  
118 incidence reached up to 80% with approximately  $10^5$  CFU of FON per gram soil in the  
119 previous growing season. The soil has a pH value of 6.15, a total nitrogen content of  
120  $0.62 \text{ g kg}^{-1}$  and available phosphorus, potassium contents of 60, 256  $\text{mg kg}^{-1}$   
121 respectively.

122 Two treatments (FOF, FBOF) and a control (OF) were set to test the effect of  
123 dazomet fumigation followed by biological organic fertilizer on watermelon Fusarium  
124 wilt disease. (I) Soil fumigated with dazomet (375 kg per ha) and treated with biological  
125 organic fertilizer (7500 kg per ha) was assigned as FBOF. (II) Soil treated with organic  
126 fertilizer (7500 kg per ha) after fumigation with dazomet (375 kg per ha) was assigned  
127 as FOF. (III) Soil treated with organic fertilizer (7500 kg per ha) without fumigation  
128 served as control (OF). Nutrients in the control and two treatments were equal.

129 The dazomet was applied into the soil evenly after soil was tilled completely. Then  
130 the soil was covered by plastic film after watered to achieve 40 % moisture. The plastic  
131 film was taken away after 10 days, which meant fumigation was terminated. Fertilizers

132 were added to the soil after another 7 days followed by another tillage. Watermelon  
133 seedlings (Sumeng NO.6) which grown to two tender buds were transplanted into the  
134 soil. Eight replicates (5 m<sup>2</sup> for each) with 10 seedlings were set in each treatment. After  
135 harvested, plants which exerted Fusarium wilt disease symptoms were counted, then  
136 soil samples were collected. In this study, the percentage of diseased plants to the total  
137 plants was computed to express the morbidity.

138

### 139 ***Soil sampling and total DNA extraction***

140

141 Soil from the top 15 cm was sampled used five-point sampling method after  
142 watermelons were harvested. Briefly, soil from five different holes were collected and  
143 mixed as one soil sample of a plot. Soil samples collected from each treatment were  
144 placed into ice box for retaining freshness then transported to the laboratory. Finally,  
145 soil total DNA was extracted under the manufacturer's protocol of the Power soil DNA  
146 Extraction kit (MOBIO Laboratories, 108 Carlsbad, CA, USA).

147

### 148 ***Soil total DNA amplification and sequencing***

149

150 Primer set 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-  
151 GGACTACHVGGGTWTCTAAT-3') (Xu et al., 2016) were utilized for the  
152 amplification of the 16S rRNA V3-V4 variable regions. ITS3F (5'-  
153 GCATCGATGAAGAACGCAGC-3') and ITS4R (5'-  
154 TCCTCCGCTTATTGATATGC-3') (White et al., 1990) were utilized for the

155 amplification of the fungal internal transcribed spacer (ITS). All the amplifications  
156 were conducted in a 50  $\mu$ L mixture including 2  $\mu$ L DNA template, 10  $\mu$ L 5 $\times$ Q5 reaction  
157 buffer, 4  $\mu$ L of 2.5 mM dNTPs, 2.5  $\mu$ L of each primer and 0.5  $\mu$ L Q5 high-fidelity DNA  
158 polymerase (New England Biolabs, UK) using Eppendorf Mastercycler nexus gradient  
159 (Eppendorf AG, Hamburg, Gemany). The conditions of Polymerase Chain Reaction  
160 (PCR) were 98  $^{\circ}$ C for 2 min, and then 35 cycles of 10 s at 98  $^{\circ}$ C, 15 s under 59  $^{\circ}$ C, 30 s  
161 at 72  $^{\circ}$ C, 72  $^{\circ}$ C for 2 min, followed by storage at 10  $^{\circ}$ C.

162

### 163 ***Bioinformatics analysis***

164

165 Sequence analyses were mainly accomplished with QIIME (Quantitative Insights  
166 Into Microbial Ecology) pipeline (Caporaso et al., 2010). Briefly, raw paired-end  
167 sequences were assembled, and then low quality and short reads were filtered. The  
168 assembled OTUs were clustered at 3 % dissimilarity and OTU tables were performed.  
169 Alpha diversity concluding Chao and Shannon indices were calculated under the OTU  
170 table. Non-Metric Multidimensional Scaling (NMDS) analysis based on Bray-Curtis  
171 distances was figured up using vegan packages in R (version 3.3.0). Manhattan plots  
172 were carried out to explore the differences between the control and treatments at the  
173 phyla level using edgeR and dplyr packages in R (version 3.3.0). Raw sequences from  
174 each sample were uploaded to the NCBI Sequence Read Archive (SRA) database  
175 (Accession number: SUB4843217).

176

177 ***Network analysis***

178

179 Phylogenetic molecular ecological networks (pMENs) of different treatments were  
180 performed on the basis of the Molecular Ecological Network Analyses Pipeline  
181 (MENAP) (<http://129.15.40.240/mena/>) (Deng et al., 2012; Zhou et al., 2010). First, a  
182 matrix of OTU-based microbial variables table file was formed as the formats required.  
183 Then, a pairwise Pearson correlation between any two OTUs was calculated by an  
184 RMT- based measure based on the abundance data. The network characteristics were  
185 determined by module detection calculations. Finally, the network graph was visualized  
186 by Gephi platform (Ling et al., 2016; Zhou et al., 2010; 2011). Zi and Pi values were  
187 calculated to represent the topological roles of each nodes and the threshold of them for  
188 classifying these OTUs were 2.5 and 0.62, respectively.

189

190 ***Statistical analysis***

191

192 Multiple comparisons between samples was carried out utilizing a one-way  
193 ANOVA with significance indicated at  $P < 0.05$  using IBM SPSS 19.0 (IBM  
194 Corporation, New York, United States). Mantel tests and pearson correlation analysis  
195 between microbial relative abundances and morbidity were performed using R (version  
196 3.3.0).

197

198 **RESULTS**

199

200 ***Disease incidence***

201

202 The watermelon Fusarium wilt incidence in control (OF) treatment reached up to  
203 100% while a lower incidence rate (29.5%) of the fumigation (FOF) treatment was  
204 observed. Compared with the control treatment, the fumigation followed by biological  
205 organic fertilizer (FBOF) treatment significantly decreased the disease incidence to 6.4%  
206 (t-test,  $P < 0.05$ ) (Fig. 1) (93.6% disease control).

207

208 ***Microbial community diversity***

209

210 The soil microbial communities (including bacteria and fungi) alpha-diversity  
211 (Chao and Shannon) indices in the fumigation (FOF and FBOF) treatments were  
212 significantly lower compared with OF treatment. However, there was no significant  
213 difference between FOF and FBOF treatments (Table 1).

214

215 Table 1 Soil microbial diversity indices

Treatment	Chao	Shannon
OF	2188±230a	6.14±0.29a

Fungi	FOF	1790±283b	5.14±1.04b
	FBOF	1782±461b	4.59±0.59b
Bacteria	OF	6730±683a	11.15±0.24a
	FOF	5227±1135b	10.44±0.74b
	FBOF	5572±891b	10.30±0.58b

216 OF: Organic fertilizer, FOF: Dazomet fumigation coupled with organic fertilizer, FBOF:  
 217 Dazomet fumigation coupled with biological organic fertilizer. Values are means ±  
 218 standard deviation (n=8). Different lowercases indicate a significant difference among  
 219 all treatments ( $P < 0.05$ ; Duncan test).

220

221 Nonmetric multidimensional scale (NMDS) analysis based on the Bray-Curtis  
 222 distance illustrated significant differences in soil microbial community composition  
 223 between the three treatments (ANOSIM,  $P < 0.05$ ) (Fig. 2). Compared to control  
 224 treatment, the microbial communities in FOF and FBOF treatments were relatively  
 225 similar.

226

### 227 ***Microbial community composition***

228

229 Under the bacterial phylum level, Proteobacteria were the most abundant,  
 230 comprising 31.3%, 35.3%, and 35.0% of the bacterial population in the OF, FOF, and  
 231 FBOF treated soils, respectively (Fig. S1). As observed, the relative abundance of

232 Firmicutes (8.5%), TM7 (5.7%), Gemmatimonadetes (5.4%), and Actinobacteria (5.3%)  
233 in FBOF treatment were significantly higher than OF and FOF treatments. Conversely,  
234 the relative abundance of Acidobacteria in FBOF treatment (2.6%) dramatic lower than  
235 that in OF (7.9%) and FOF (4.1%) treatments.

236 The relative abundance of the fungi *Arthrobotrys* (16.5%) and *Phialemonium*  
237 (8.1%) in FBOF treatment were significantly higher compared to OF and FOF  
238 treatments (Fig. S1). The genus *Trichoderma* accounted for 2.2%, 10.8% and 13.5% of  
239 the fungal community in the OF, FOF and FBOF treatments, respectively. The relative  
240 abundance of *Fusarium* in OF (7.9%) was the highest among the three treatments.  
241 Compared to OF, the FBOF treatment significantly increased the abundance of  
242 Firmicutes (Fig. S2), *Bacillus*, and *Trichoderma* (Fig. 3).

243

244 A Mantel test indicated that the bacterial ( $\rho = 0.30$ ,  $P < 0.01$ ) and fungal ( $\rho =$   
245  $0.52$ ,  $P < 0.01$ ) community compositions were significantly correlated with disease  
246 incidence (Table S1). Among the dominant microbial genera (relative abundance  $>1\%$ ),  
247 *Bacillus* ( $r = -0.56$ ,  $P = 0.004$ ) and *Trichoderma* ( $r = -0.56$ ,  $P = 0.006$ ) illustrated  
248 significant negative correlations with *Fusarium* relative abundance (Fig. 3).

249

250 ***Different effects of amendments on soil microbial molecular ecological network***

251

252 Network plots revealed varying microbial co-occurrence network structures among  
253 the three treatments (Fig. 4). Different  $R^2$  values of 0.835, 0.951 and 0.918 for FBOF,  
254 FOF and OF treatments, respectively, indicating networks formed possess scale-free  
255 properties (Table. S2). With more links in FBOF treatment than FOF treatment, the  
256 biological organic fertilizer application after fumigation increased the connectivity,  
257 resulting in a more complex soil microbial community.

258

259 The topological character of each nodes was demonstrated with Zi-Pi plot (Fig. S3).  
260 Majority (96.4%) of the nodes were peripherals as most of their links inside their  
261 modules. About 3.5% of the nodes were module hubs while only one Bacteroidetes  
262 OTU was a connector. Among all the module hubs, a total of 43, 6, and 24 OTUs  
263 belonged to the OF, FOF and FBOF treatments, respectively. Three of the 24 FBOF  
264 module hubs were Firmicutes, and two of them were closely related to *Bacillus*. Three  
265 module hubs in FBOF were assigned to Ascomycota, of which two were closely related  
266 to *Trichoderma*. The other hubs belonged to varying taxa (i.e., Proteobacteria,  
267 Gemmatimonadetes, Chloroflexi, and Acidobacteria) (Table S3).

268

## 269 **DISCUSSION**

270

271 As we aimed to explore the underlying mechanisms that link dazomet fumigation  
272 followed by biological organic fertilizer application with watermelon Fusarium wilt

273 disease suppression. Two fumigation treatments, especially FBOF (applied biological  
274 organic fertilizer after fumigation), were effective in reducing Fusarium wilt disease  
275 incidence. This demonstrated that dazomet fumigation was highly effective in  
276 suppressing Fusarium wilt disease, as supported by previous studies (Tian et al., 2014).  
277 In the meantime, treatment with only organic fertilizer (OF) resulted in the highest  
278 disease incidence, demonstrating that organic fertilizer application alone was not  
279 effective in inducing disease suppression in severe diseased area. A previous study  
280 indicated that the application of organic fertilizer alone was ineffective and possibly  
281 propitious to the growth of pathogens (Bonanomi et al., 2010). Thus, the utilization of  
282 dazomet followed by a biological organic fertilizer amendment was a better alternative  
283 for controlling Fusarium wilt disease.

284 It has been generally considered that there exists three possible ways in which the  
285 soil microbial community may not show response to a disturbance. (I) The soil  
286 microbial community may be resistant to the disturbance; (II) the microbial community  
287 suffered the disturbance and then perform a resilient, allowing a rapid recover to its  
288 intrinsic status; (III) even if the microbial community composition is altered by the  
289 disturbance, the new community may be functionally similar to its intrinsic status  
290 (Allison and Martiny, 2008). In our greenhouse study, we observed that the microbial  
291 communities were principally sensitive to dazomet fumigation and resilient to the  
292 disturbance caused by the combination of dazomet fumigation and bio-fertilizer  
293 application. In comparison to organic fertilizer amendment alone (OF), both the Chao

294 and Shannon indices exhibited dramatic decreases in microbial richness and diversity  
295 in the fumigated treatments. The result is identical with previous findings that  
296 fumigation could reduce microbial biodiversity (Fu et al., 2012; Griffiths et al., 2000).  
297 The control treatment exhibited significantly higher richness and abundance than the  
298 dazomet-treated samples further supporting the impact of fumigation, as dazomet is  
299 widely known to suppress the growing of microbial communities (Eo and Park, 2014;  
300 Scopa and Dumontet, 2007). On the basis of the insurance hypothesis, biodiversity loss  
301 might lead to a reduction in ecosystem stability (Griffiths and Philippot, 2013).  
302 Previous experiments have supported the hypothesis by using differential gradient  
303 fumigation to decrease soil biodiversity, and the lower biodiversity in soil indicated a  
304 reduced resilience in plant decomposition after disturbance (Griffiths et al., 2000).

305 It seems that the compositional transformation of the intrinsic *Fusarium*-dominated  
306 soil microbial community to a re-assembled non-disease community was the basis for  
307 disease suppression (van Elsas et al., 2012). Supporting this speculation, we observed  
308 the bacterial and fungal community composition of OF treatment was distinctly  
309 different from the other two treatments (FOF, FBOF) which supports the differences in  
310 the alpha-diversities between the treatments. This confirms previous results where  
311 fumigation often lead to diversification in community composition (Ibekwe et al., 2001;  
312 Omirou et al., 2010; Shen et al., 2018). In addition, the initial disturbance by fumigation  
313 was able to break the stability of soil microbial community by decreasing community  
314 diversity and alter the community structure and potentially releasing the available niche

315 space, temporarily decreasing species competition. Then the invader whether colonized  
316 in the soil depends on its ability to achieve the available niche space within the disturbed  
317 community (Kinnunen et al., 2016).

318 Two types of fertilizer in our greenhouse study are composts rich C, N, and  
319 exogenous microbes (*Bacillus* and *Trichoderma* in bio-fertilizer). When applied to soil,  
320 they advance the soil fertility, ameliorate soil structure and increase or decrease several  
321 microorganisms abundances and activities (Ling et al., 2014; Suleiman et al., 2016).  
322 Hence, different amendments endowed distinct microbial communities. Lower  
323 abundance of the pathogen *Fusarium* and higher abundances of the antagonistic agents  
324 *Bacillus* and *Trichoderma* introduced by biological organic fertilizer are likely related  
325 to *Fusarium* wilt disease incidence in this study. Mantel tests revealed that microbial  
326 community composition might be a key factor in disease suppression. The highest  
327 relative abundance of Firmicutes identified within the FBOF treatment revealed that  
328 they may be involved in disease suppression in these soils (Trivedi et al., 2017; Xiong  
329 et al., 2017b). The Firmicutes include numerous potential biocontrol agents and has  
330 been reported with a higher abundance in suppressive soils of different soil-borne  
331 disease systems (Rosenzweig et al., 2012; Shen et al., 2015). At the genus level,  
332 *Bacillus*, which was widely used to suppress soil-borne wilt disease, were significant  
333 higher in FBOF than other two treatments (Cao et al., 2011; Zhang et al., 2008). Due  
334 to their broad-spectrum antibiotic activity and the ability of form endospores, the  
335 *Bacillus* species possess several advantages in excess of other agents for protection

336 against pathogens (Cavaglieri et al., 2005). Previous research on cucurbits showed that  
337 fumigation can kill the mycelia of *F. oxysporum* of cucumber and then result in a  
338 dramatic abundance diminution of the pathogen (Li et al., 2016). For further impact  
339 restrained the relative abundance of *Fusarium*, biological organic fertilizer application  
340 build on the initial express of fumigation, which due to the disease suppressive capacity  
341 of microbial populations contained within the biological organic fertilizer. It should be  
342 noticed that pathogenic or non-pathogenic *Fusarium* were not separately quantified in  
343 this study yet. Nevertheless, field disease incidence revealed that the pathogenic  
344 *Fusarium* played a main role in the control treatment. Besides, the *Fusarium* population  
345 was very low in the fumigated treatment, so this might not be an issue. As a kind of  
346 biological control agent, the genus *Trichoderma* could compete nutrients and space  
347 with pathogens, alter soil conditions, promote the growth of plant, or some direct  
348 biocontrol such as mycoparasitism and antibiosis to exert biocontrol against fungal  
349 pathogen (Benítez et al., 2004). These beneficial species (*Bacillus* and *Trichoderma*)  
350 introduced by bio-fertilizer were detected at increased relative abundances in the FBOF  
351 treatment. Linear models revealed that higher relative abundance of  
352 *Bacillus/Trichoderma* might generate negative effect on *Fusarium* relative abundances.  
353 Therefore, the colonization of introduced *Bacillus* and *Trichoderma* after fumigation in  
354 our experiment had a direct effect on pathogen and, consequently, on the suppression  
355 of *Fusarium* wilt disease.

356 Several previous studies have illustrated that the biological organic fertilizer  
357 amendment played a major role in shaping soil microbial community composition  
358 (Ling et al., 2014; Qiu et al., 2012). Our results demonstrated that empty niches created  
359 due to fumigation are filled by microbial populations introduced by biological organic  
360 fertilizer which leads to interactions with the surviving resident populations. In this  
361 manner, the remodeling of the soil microbiome, in concert with potential antagonistic  
362 capacities, serve to suppress plant disease (Akhtar and Malik, 2000).

363 Microbial diversity contains not only the abundance of species but also the  
364 complex interactions among different species (Olesen et al., 2007). Based on ecosystem  
365 theory, the sensibility of soil ecosystems to invader rest with their complexity  
366 (Fließbach et al., 2009). The use of phylogenetic molecular ecological networks  
367 (pMENs) is one of the methods to investigate the complexity of interactions within a  
368 microbial community. The fumigated (FOF and FBOF) treatments exhibited a lower  
369 number of nodes and links compared with OF treatment, due to the reduced soil  
370 microbial community complexity and stability after fumigation. However, compared to  
371 the FOF treatment, the FBOF treatment exhibited a higher number of nodes and links  
372 and a higher ratio of positive/ negative links. Our results indicated that the re-shaped  
373 microbial community resulted in more microbial cooperation than competition.

374 Each soil microbial ecological network is made up of a series of nodes, and each  
375 node has a different role in the network topological structure. Although the number of  
376 module hubs in fumigation treatments was under non-fumigated treatment, the FBOF

377 treatment contained more module hubs than FOF. This suggests that even though  
378 fumigation decreased the overall number of interactions, the biological organic  
379 fertilizer amendment resulted in a less drastic decrease in network complexity than  
380 organic fertilizer alone. Previous theory predicts that a group of species with higher  
381 probability of interacting with each other shows higher resistance and resilience against  
382 distribution owing to their buffering of the extinctions (Stouffer and Bascompte, 2011).  
383 The majority of the module hubs in the FBOF treatment were bacteria, indicating that  
384 biological organic fertilizer amendment stimulated the microbial network to recover  
385 towards a bacterial- dominant community. Our previous research has demonstrated that  
386 *Fusarium* wilt disease may be more susceptible in fungal- dominant microbial  
387 communities (Zhao et al., 2017).

388       Only three module hubs were identified as fungi. Two of these were identified as  
389 the genus *Trichoderma* and the other one (OTU fun3928) was identified within the  
390 order Hypocreaceae. *Trichoderma* belong to the order Hypocreaceae, thus OTU  
391 fun3928 may have similar functions in *Fusarium* disease suppression as *Trichoderma*.  
392 However, additional research is necessary to support this hypothesis. Two module hubs  
393 within the FBOF treatment were assigned to *Bacillus*. Overall, our results indicate that  
394 the introduced species (*Bacillus* and *Trichoderma*) within the biological organic  
395 fertilizer which were significantly negatively correlated to *Fusarium* relative  
396 abundance, may be pivotal in the restoration of a suppressive soil microbial community.

397 A graphical and conceptual interpretation of the mechanism under the observed  
398 results is therefore hypothesized (Fig. 5). The initial disturbance by dazomet destroy  
399 the competition between numerous microbial taxa, which touches off a competitive  
400 release then exogenous species can more easily occupy the free niches. Consequently,  
401 the soil microbial community composition is re-arranged, and the niche differentiation  
402 pattern of the resident community is altered. This alteration in the community  
403 composition with biological organic fertilizer amendment then results in the  
404 establishment of a disease-suppressive community that acts through a combination of  
405 direct and indirect antagonism towards the pathogen.

406 In conclusion, the result of this study shows that dazomet fumigation followed by  
407 biological organic fertilizer application effectively controlled watermelon *Fusarium*  
408 wilt disease, and is likely attributed to a combination of both the direct suppression of  
409 the pathogen population and the re-shaping of the soil microbiome. Fumigation  
410 disturbance resulted in a strong reduction in microbial community diversity. This  
411 appears to result in niche release and the drastic reduction in interactions within the soil  
412 microbial community. The beneficial species (*Bacillus* and *Trichoderma*), introduced  
413 through biological organic fertilizer amendment re-colonize the vacant niches and,  
414 through their interactions, manipulate the composition of the soil microbial community.  
415 This promotes a reversion to a relatively complex microbial community. Thus, the  
416 overall effectiveness of fumigation followed by biological organic fertilizer application  
417 appears to be a combination of introduced microbial direct effects on *Fusarium* and the

418 promotion of changes in microbial community composition promoted by the biological  
419 organic fertilizer (Fig. 6). Therefore, we propose that manipulating the community re-  
420 assembly that occurs after fumigation is essential to promote sustainable plant health in  
421 the face of a growing worldwide pathogenic threat.

#### 422 **Ethics approval and consent to participate**

423 Not applicable

#### 424 **Consent for publication**

425 Not applicable

#### 426 **Availability of data and materials**

427 All data generated or analysed during this study are included in this published  
428 article (and its supplementary information files).

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#### 436 **Authors' contributions**

437 This study was conceived and led by Qirong Shen. Xu Zhang, Dan Fang,  
438 Xiaohui He and Mengyu Wei did the field experiment and collected the samples. Xu  
439 Zhang, Chenjin Zhuo and Junyao Jin finished all the lab work. Xu Zhang and Chao

440 Xue analyzed the data and wrote the manuscript. All authors discussed the results and  
441 their implications and commented on the manuscript as it progressed.

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628

629 **Figure legend**

630 **Fig. 1** Disease incidence of watermelon Fusarium wilt in different treatments.  
631 OF: Organic fertilizer, FOF: Dazomet fumigation followed by organic fertilizer, FBOF:  
632 Dazomet fumigation followed by biological organic fertilizer. Different lowercases  
633 indicate a significant difference at the 0.05 probability level according to the Duncan  
634 test.

635 **Fig. 2** Nonmetric Multidimensional scaling (NMDS) analysis in bacterial (A) and  
636 fungal (B) microbiome between all sample sets.  
637 OF: Organic fertilizer, FOF: Dazomet fumigation followed by organic fertilizer, FBOF:  
638 Dazomet fumigation followed by biological organic fertilizer.

639 **Fig. 3** The relative abundance (RA) of genus *Bacillus* (A) and *Trichoderma* (B) in the  
640 three treatments and Pearson correlations (r) between RA of *Bacillus* (C) and  
641 *Trichoderma* (D) with RA of *Fusarium*.  
642 OF: Organic fertilizer, FOF: Dazomet fumigation followed by organic fertilizer, FBOF:  
643 Dazomet fumigation followed by biological organic fertilizer.

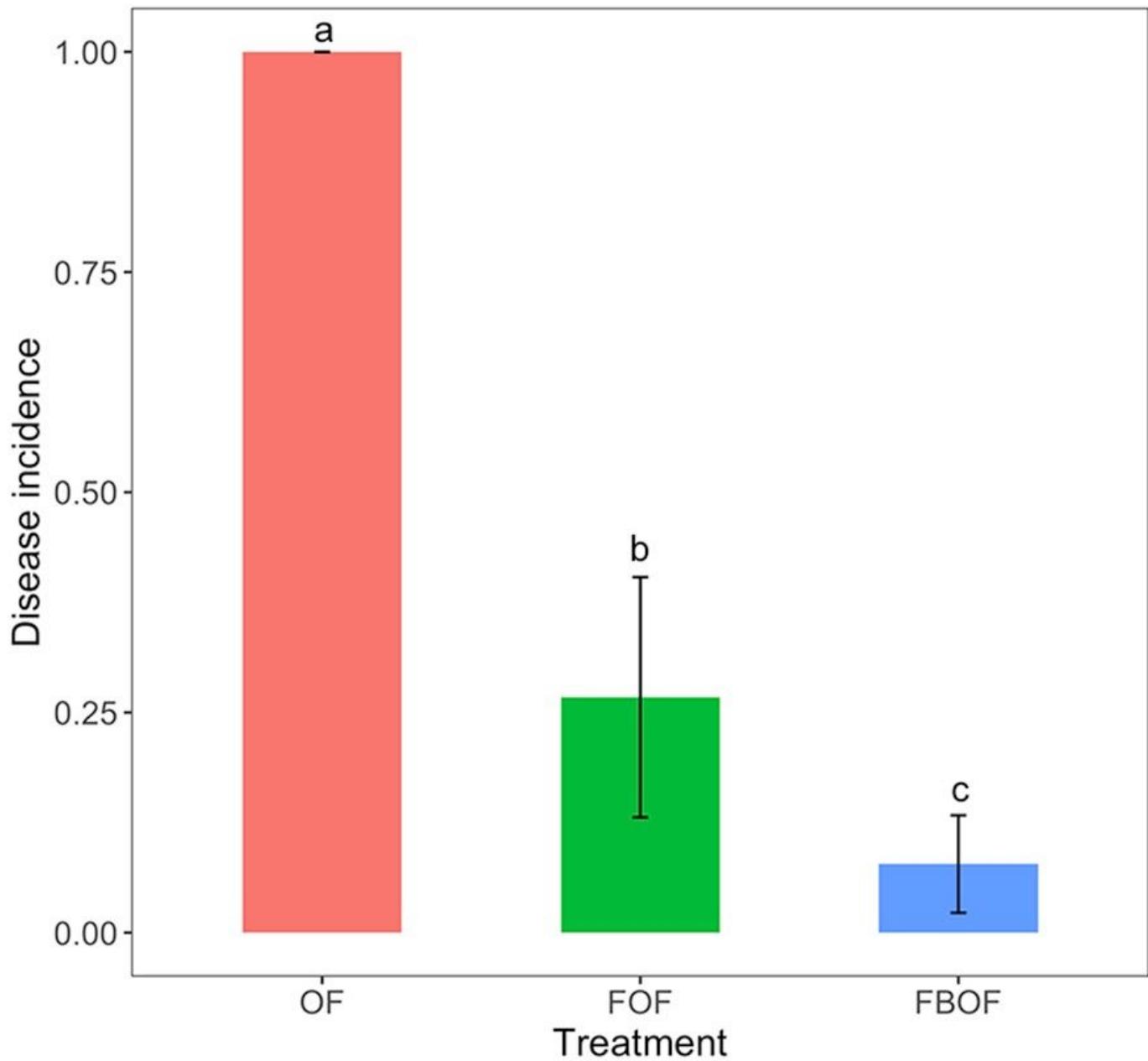
644 **Fig. 4** Different networks performed by OTU tables based on RMT analysis.  
645 OF: Organic fertilizer, FOF: Dazomet fumigation followed by organic fertilizer, FBOF:  
646 Dazomet fumigation followed by biological organic fertilizer. Different networks  
647 represent random matrix theory co-occurrence models for each treatment. Different  
648 nodes mean different OTUs, and links between the nodes indicate significant  
649 correlation. Modules are randomly colored for, and modules with more than 5 nodes  
650 were shown.

651 **Fig. 5** A conceptual model for understanding fumigation followed by biological organic  
652 fertilizer impacts on soil resident microbial community. Consider a microbial  
653 community of resident soil as a circle, as shown in (A). When dazomet is applied into  
654 the soil, the niches in resident soil are released (B). The introduced species in biological  
655 organic fertilizer will take up the niches (C) and alter the niche structure in such way  
656 (D).

657 **Fig. 6** A conceptual cartoon summarizing the influence of dazomet followed by  
658 biological organic fertilizer treatments on soil microbial community and their influence  
659 on watermelon Fusarium wilt disease suppression.

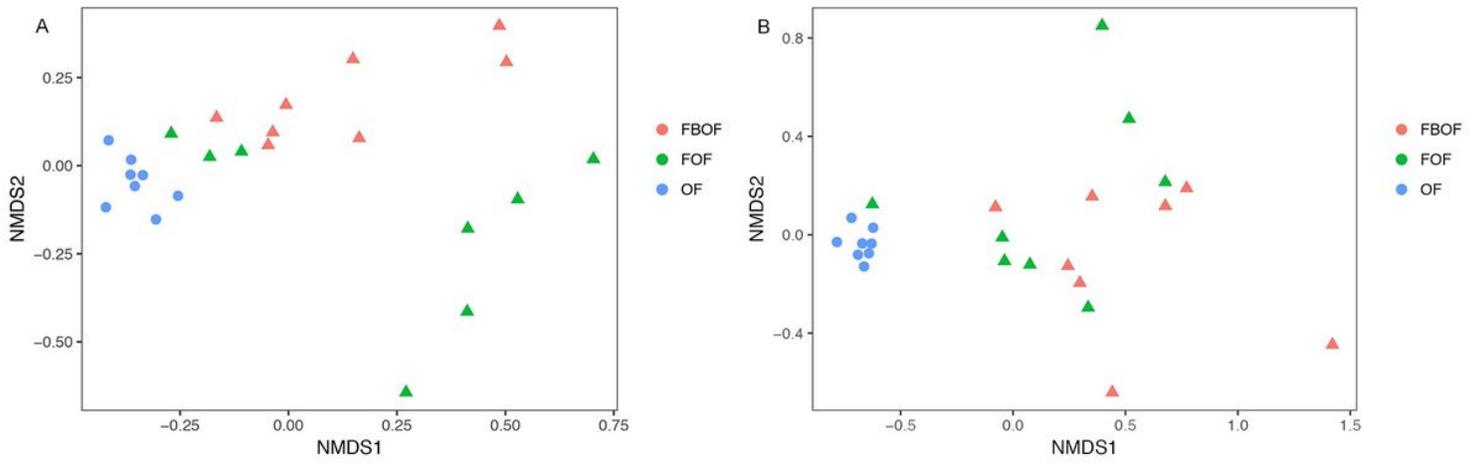
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## Figures



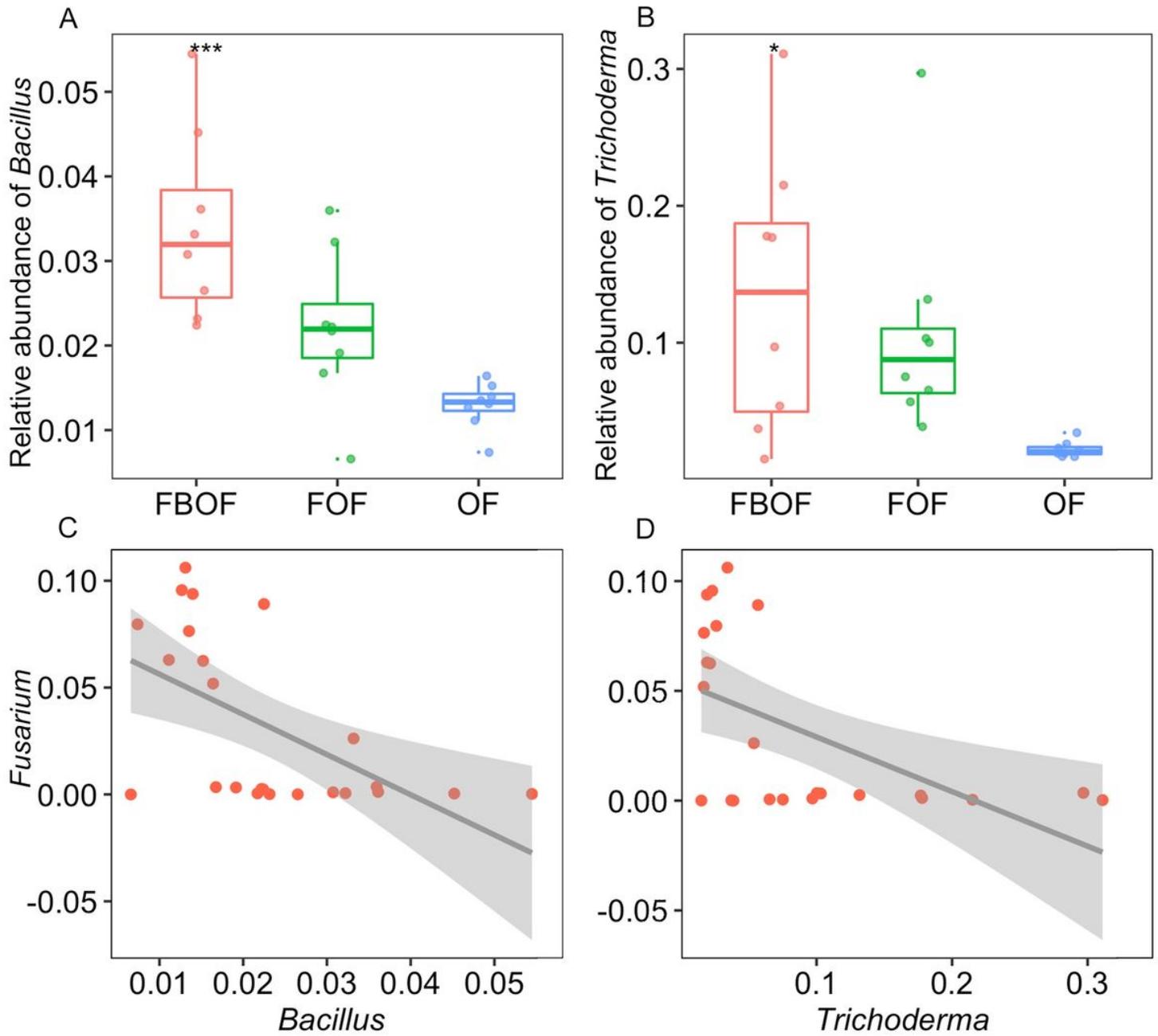
**Figure 1**

Disease incidence of watermelon Fusarium wilt in different treatments. OF: Organic fertilizer, FOF: Dazomet fumigation followed by organic fertilizer, FBOF: Dazomet fumigation followed by biological organic fertilizer. Different lowercases indicate a significant difference at the 0.05 probability level according to the Duncan test.



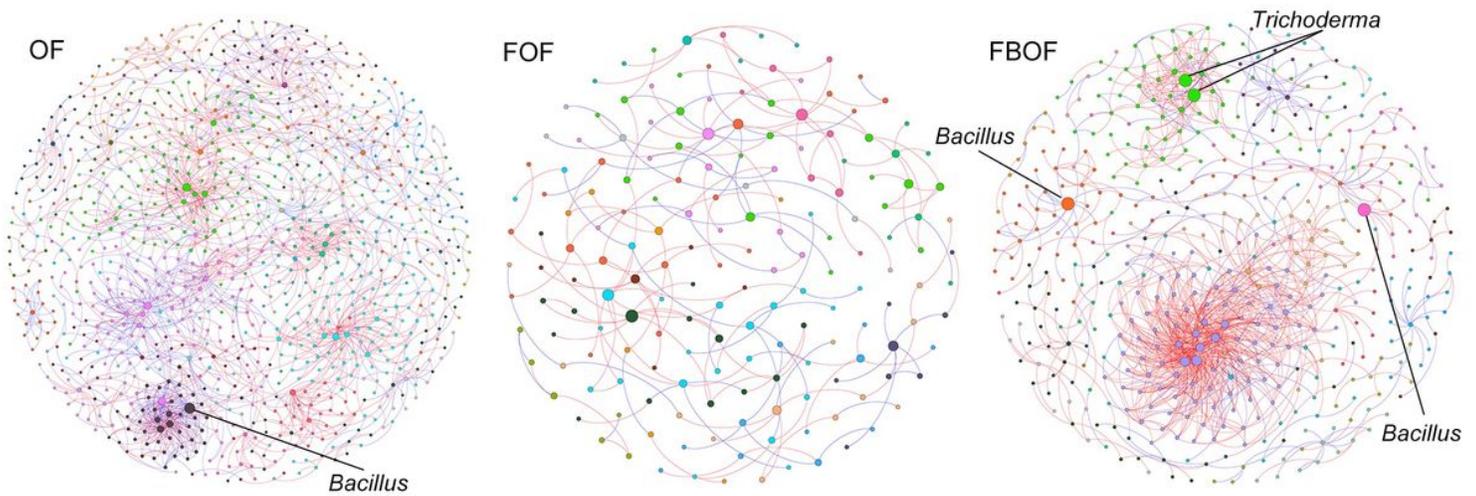
**Figure 2**

Disease incidence of watermelon Fusarium wilt in different treatments. OF: Organic fertilizer, FOF: Dazomet fumigation followed by organic fertilizer, FBOF: Dazomet fumigation followed by biological organic fertilizer. Different lowercases indicate a significant difference at the 0.05 probability level according to the Duncan test.



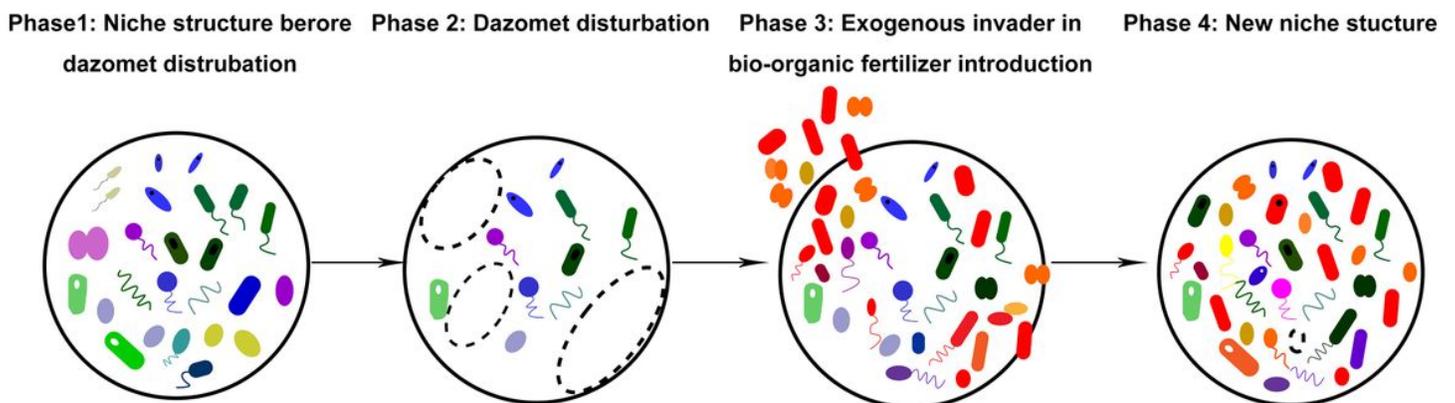
**Figure 3**

The relative abundance (RA) of genus *Bacillus* (A) and *Trichoderma* (B) in the three treatments and Pearson correlations (r) between RA of *Bacillus* (C) and *Trichoderma* (D) with RA of *Fusarium*. OF: Organic fertilizer, FOF: Dazomet fumigation followed by organic fertilizer, FBOF: Dazomet fumigation followed by biological organic fertilizer.



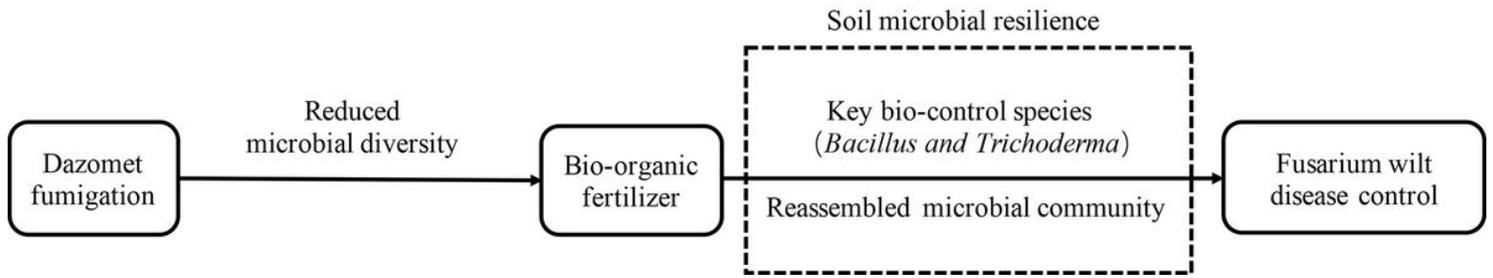
**Figure 4**

Different networks performed by OTU tables based on RMT analysis. OF: Organic fertilizer, FOF: Dazomet fumigation followed by organic fertilizer, FBOF: Dazomet fumigation followed by biological organic fertilizer. Different networks represent random matrix theory co-occurrence models for each treatment. Different nodes mean different OTUs, and links between the nodes indicate significant correlation. Modules are randomly colored for, and modules with more than 5 nodes were shown.



**Figure 5**

A conceptual model for understanding fumigation followed by biological organic fertilizer impacts on soil resident microbial community. Consider a microbial community of resident soil as a circle, as shown in (A). When dazomet is applied into the soil, the niches in resident soil are released (B). The introduced species in biological organic fertilizer will take up the niches (C) and alter the niche structure in such way (D).



**Figure 6**

A conceptual cartoon summarizing the influence of dazomet followed by biological organic fertilizer treatments on soil microbial community and their influence on watermelon *Fusarium* wilt disease suppression.

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