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An assessment of the modulation of the population dynamics of pathogenic *Fusarium oxysporum* f. sp. *lycopersici* in the tomato rhizosphere by means of the application of *Bacillus subtilis* QST 713, *Trichoderma* sp. TW2 and two composts

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(Article begins on next page)

1 **Research paper**

2 **An assessment of the modulation of the population dynamics of pathogenic *Fusarium***
3 ***oxysporum* f. sp. *lycopersici* in the tomato rhizosphere by means of the application of *Bacillus***
4 ***subtilis* QST713, *Trichoderma* sp. TW2 and two other composts**

5

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18 **Running head: Biological Control of Tomato Fusarium Wilt with BCAs and composts**

19

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27 **Abstract**

28 Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *lycopersici* (Fol), results in considerable yield
29 losses for tomato crops throughout the world. In this study, experiments were carried out “*in situ*”
30 over two consecutive years to evaluate the efficiency of two biological control agents (BCAs)
31 (*Bacillus subtilis* QST713 and *Trichoderma* sp. TW2) and two composts in controlling the disease.
32 In this context, the quantitative polymerase chain reaction was used along with soil chemical
33 parameters to study the general effects of treatments on the severity of the disease and on the non-
34 target microbial populations residing in the studied rhizosphere and bulk soil. The ecological fitness
35 of the BCAs was also evaluated. Furthermore, as BCAs produce elicitors which may activate plant
36 defense reactions, particular attention was paid to the induction of pathogenesis-related genes (PR)
37 in the roots of tomato plants.

38 The preventative nursery application of all three types of biocontrol agents, that is, *Bacillus subtilis*
39 QST713, *Trichoderma* sp. TW2 and compost, as separate treatments, induced a significant
40 reduction in the disease, compared to the untreated control, and reduced tomato Fusarium wilt by
41 70%. This result was confirmed by the significant negative correlations between the abundance of
42 biological control agents and the severity of the disease. In general, the BCA and compost
43 treatments did not induce a negative effect on the non-target microbial communities. The transcript
44 levels coding for the studied pathogenesis-related (PR) genes were always higher in the presence of
45 Fol on its own (untreated control) for all genes considered. However, the accumulation of
46 transcripts in the tomato roots was different, depending on the treatment. An important level of
47 disease reduction was shown by a decrease in Fol abundance, together with a greater abundance of
48 the inoculated BCA populations and an accumulation of transcripts encoding PR genes.

49 In short, the results of this study reinforce the concept of the sustainability of treatments based on
50 biological control agents and composts for the management of tomato Fusarium wilt.

51

52 **Keywords:** *Fusarium oxysporum* f.sp. *lycopersici* (Fol); BCAs; disease suppression; pathogenesis

53 related genes (PR)

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78 **1. Introduction**

79 Tomato (*Lycopersicon esculentum* Mill.) is among the world's most important vegetables, with a
80 global annual yield of approximately 160 million tons for all types of tomatoes (FAO, see the
81 statistical database [FAOSTAT], 2014; available on: <http://fatstat.fao.org>). According to Lopes and
82 Ávila (2005), about 200 biotic or abiotic diseases have been reported on tomato throughout the
83 world. *Fusarium oxysporum* f. sp. *lycopersici* (Fol) is a soil-borne pathogen that causes the
84 Fusarium wilt of tomato, which results in severe production losses (Gale et al., 2003; Huang et al.,
85 2012; McGovern, 2015).

86 The eradication of soil-borne pathogens (including Fol) is generally based on an integrated
87 management programme, consisting of chemical soil fumigation and the use of resistant cultivars
88 when available (McGovern, 2015), but also on the use of organic amendments (Dukare et al., 2011;
89 Pugliese et al., 2008; 2010) and biocontrol agents (BCAs) (Muslim et al., 2003; Gowtham et al.,
90 2016; Huang et al., 2012; Jangir et al., 2018). *Bacillus* spp. and *Trichoderma* spp., which are
91 available as commercial and experimental products, are among the most frequently used BCAs
92 today (Jacobsen et al., 2004; Lorito and Woo, 2015). They are abundant in soil and have been
93 investigated for their potential application as BCAs against several plant pathogens, including Fol
94 (Cotxarrera et al., 2002; de Medeiros et al., 2017; Ghazalibiglar et al., 2016; Lorito et al., 2010;
95 Zhao et al., 2014). The combination of BCAs with organic amendments (e.g., compost, manure,
96 plant waste) has also been studied to control soil-borne plant pathogens (Borrero et al., 2004, 2012;
97 Cotxarrera et al., 2002; Pugliese et al., 2011).

98 BCA activities include the secretion of secondary metabolites (such as cyclic lipopeptides and
99 volatile organic compounds), and competition for nutrients and parasitism (Kim et al., 2015; Vitullo
100 et al., 2012). In addition to these antagonistic effects on plant pathogens, BCAs produce elicitors
101 that activate plant defence reactions (Walters and Daniell, 2007), such as the induction of
102 pathogenesis-related (PR) genes (Edreva, 2005) encoding chitinase and β -1,3-glucanases. These
103 two groups of PR proteins have attracted considerable interest because of their “*in vitro*” and “*in*

104 *vivo*” inhibitory activity against fungi (Van den Elzen et al., 1993; Bargabus et al., 2002). In
105 addition, PR proteins (glucanases and chitinase) have been seen to increase concomitantly with the
106 development of the disease (Rep et al., 2002). For example, it has been acknowledged that the
107 treatment of sugar beet with *Bacillus mycoides* induces the expression of PR genes such as β -1,3-
108 glucanase (Bargabus et al., 2002). As far as the inoculation of tomato plants with *F. oxysporum* f.
109 sp. *Lycopersici* is concerned, increases in chitinase, β -1,3-glucanase, and β -1,4-glucosidase
110 activities have been observed to be lower in resistant cultivars than in susceptible ones (Ferraris et
111 al., 1987). However, relatively little is known about the effect of BCA and compost as pre-planting
112 treatments on the accumulation status of PR genes in plants at the end of the cropping cycle in the
113 correspondences with the peak of the disease (Borrero et al., 2004).

114 BCAs are known to colonise the rhizosphere, thereby indirectly affecting the microbial processes
115 that are essential for the general functioning of the soil ecosystem. This rhizosphere colonization
116 includes effects on the resident microorganisms and in consequence on carbon, nitrogen and
117 phosphorus cycling (Bonanomi et al., 2010; Gupta et al., 2012). This may result in adverse effects
118 on crop yield and health. Hence, there is a need to study the potential side - effects of the applied
119 treatments on the indigenous rhizosphere and soil microbial populations (Bonanomi et al., 2018).

120 The main objectives of this study were: i) to evaluate the effect of two BCAs and two composts on
121 the severity of the fusarium wilt of tomato and on the non-target rhizosphere and bulk soil microbial
122 communities in the tomato - *Fusarium oxysporum* f.sp. *lycopersici* (Fol) pathosystem, in a naturally
123 infested soil; ii) to obtain new insights into the induction of pathogenesis-related (PR) genes in pre-
124 planting treated tomato roots at the end of the tomato crop cycle, which usually coincides with the
125 highest disease incidence. Therefore, the differential expression of genes encoding *GluA* (acidic
126 extracellular b-1,3-glucanase), *GluB* (basic intracellular b-1,3-glucanase), *Chi3* (acidic extracellular
127 chitinase), *Chi9* (basic intracellular chitinase) and *PR-1a* was studied.

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129

130 2. Materials and Methods

131 2.1. Layout of the experiments

132 Two experiments (2016 and 2017) were carried out in plastic tunnels on a commercial farm in Asti
133 (Northern Italy), which had a history of several tomato cycles prior to the beginning of this study, in
134 a sandy loam soil (sand : silt : clay 50 : 40 : 10 %, with neutral pH and 1.5 % organic matter),
135 naturally infested with Fol.

136 The experimental trials were carried out over two consecutive years, 2016 and 2017, in order to test
137 the efficacy of pre-planting soil treatments with a commercially available formulation of *Bacillus*
138 *subtilis* QST713 - (SM, Serenade Max, Bayer Crop Science, Italy) and an experimental BCA
139 (*Trichoderma* sp., TW2, AgriNewTech, Italy) (Table 1) against Fol. In addition, two commercial
140 composts that is Ant's Compost V and Ant's Compost M; AgriNewTech, Italy, produced from
141 green wastes in a dynamic industrial treatment system, were used (Table 1). Ant's Compost M is in
142 fact Ant's Compost V inoculated with *Trichoderma* sp. TW2. An untreated control was used to
143 monitor Fusarium wilt development. Summarised details of these treatments can be found in Table
144 1.

145 Tomato seeds (cv. Ingrid, Seminis) were sown in 40-plug trays (53 x 42 cm; plug 10x10 cm, 4l peat
146 capacity) filled with a peat mixture substrate.

147 Four soil treatments with BCAs were carry out on the plug tray between sowing and transplanting
148 in a greenhouse in a commercial nursery (Table 1). The BCAs were applied by spraying them onto
149 the soil surface in a high volume of water (400 ml/tray) using a 1 l capacity hand sprayer. The
150 application times were: 37 days after sowing, which was defined as T1, for the first treatment, 44
151 days after sowing, defined as T2, for the second treatment, 51 days after sowing, defined as T3, for
152 the third treatment and 58 days after sowing, defined as T4, for the fourth treatment. The compost
153 products were applied twice: they were first mixed with the substrate at sowing, and this
154 combination was defined as T0, and they were then mixed with the soil one week before

155 transplanting at T3, that is, 51 days after sowing, according to dosages and application times
156 reported in Table 1.

157 The tomato seedlings at the fourth true leaf stage were transplanted (T58) into naturally Fol infested
158 soil at a density of 2.5 plants/m², drip irrigated and then grown according to the cultural practices
159 adopted by commercial growers in the region. The plots were arranged in a completely randomised
160 block design, with four replicates per treatment. The plants were monitored every 10-15 days to
161 determine the development of symptoms. Disease severity (DS) data were recorded, starting from
162 the appearance of the first symptoms (yellow leaves and reduced growth). The final disease rating
163 was carried out 128 days after transplanting, on 16-20 plants per treatment by dissecting each plant.
164 For this purpose, a DS scale was created as follows: 0 = healthy plant, 25% = initial leaf chlorosis,
165 50% = severe leaf chlorosis and initial symptoms of wilting during the hottest hours of the day,
166 75% = severe wilting and severe symptoms of leaf chlorosis; 100% = plant totally wilted, leaves
167 completely necrotic (Borrero et al., 2004; Srinivasan et al., 2009).

168

169 2.2. Rhizosphere and bulk soil sampling

170 The effect of the two BCAs and composts on indigenous microbial communities was studied at the
171 rhizosphere and bulk soil levels by sampling at the end of the trials (2016 and 2017), as described
172 by Cucu et al., (2018). In brief, rhizosphere and bulk soil were collected, with three biological
173 replicates per treatment. Each replicate consisted of five sub-replicates, which were pooled together.
174 The replicate samples were collected in a W - shaped sampling pattern from each plot throughout
175 the field, in order to ensure a good bulk soil homogeneity. The dry weight of the roots was also
176 determined.

177 The fresh rhizosphere and bulk soil samples were accurately homogenized separately, sieved
178 through a 2 mm sieve and stored at 4°C. All the samples were split for further geochemical
179 analysis: humidity, pH, total N (TN), inorganic N as nitrate (NO₃⁻) and ammonium (NH₄⁺),

180 dissolved organic carbon (DOC), total phosphorus (Pt) and available phosphorus (Pav), as well as
181 for molecular investigations.

182

183 2.3. Molecular analyses

184 2.3.1. Rhizosphere and bulk soil DNA extraction

185 A NucleoSpin® Soil kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) was used,
186 according to the manufacturer's instructions, for the rhizosphere and bulk soil genomic DNA
187 extraction. The extraction was conducted on fresh samples (250-500 mg of sample material). The
188 quantity and purity of the DNA were measured spectrophotometrically using a NanoDrop ND-1000
189 device (NanoDrop Technologies, Wilmington, DE, USA).

190

191 2.3.2 Microbial abundance (quantitative PCR (qPCR) assays)

192 The abundance of the bacterial and archaeal 16S rRNA genes, fungal 18S rRNA genes and of three
193 functional genes, (i.e., bacterial ammonia monooxygenase *amoA* - AOB, archaeal *amoA* - AOA and
194 fungal chitinase - *chiA* was determined by means of qPCR, using a StepOnePlus™ Real-Time PCR
195 System (Applied Biosystems, Foster City, CA, USA) for the rhizosphere and bulk soil DNA
196 samples. In addition, the abundance of *Fol*, *Bacillus* spp. and *Trichoderma* spp. was also
197 investigated. A description of the primer sets and amplification details is given in Table 2.

198 Amplicons were generated from each target gene for the standard preparation, purified (Invisorb
199 Fragment CleanUp, Stratec Molecular GmbH, Berlin, Germany) and ligated in a Strata-Clone PCR
200 cloning vector pSC-A (Strataclone PCR Cloning Kit, Agilent Technologies Inc.); the ligation
201 products were then transformed using StrataClone SoloPack competent cells (Agilent Technologies
202 Inc.). The specificity of the clones used as qPCR standards was checked, via sequencing, at LGC
203 Genomics GmbH (Berlin, Germany), and through BLAST analysis. Plasmid DNA was isolated
204 (GenElute™ Plasmid Miniprep Kit, Sigma-Aldrich, St. Louis, MO, USA) from standard clones and
205 quantified as described above.

206 After the qPCR assay optimisation, the final volume of the qPCR reactions was established as 25 μ l
207 for the 16S and 18S rRNA genes, for the bacterial and archaeal *amoA*, and for the Fol and BCA
208 quantification, while it was 20 μ l for *chiaA*. All the qPCR assays were performed using 10 ng DNA
209 as a template, except for the bacterial 16S rRNA gene, for which 5 ng DNA was used (Cucu et al.,
210 2017; 2018). The reaction mixtures contained a 1x Power SYBR green master mix (Applied
211 Biosystems), with 0.12 μ M of each oligonucleotide (Table 2) for the total bacteria, archaea, fungi
212 and Fol, 0.32 μ M for the AOB, AOA and BCAs and 0.4 μ M for *chiaA*.

213 All the considered genes were quantified in triplicate across the plates, while standards were run in
214 duplicate in 10-fold serial dilutions. The amplification efficiency ranged from 96%, (archaeal 16S
215 rRNA, fungal 18S rRNA, BCAs and their functional genes) to 103% (bacterial 16S rRNA). The
216 amplification efficiency of the nitrifiers was 95.3% and 99.1% for AOB and AOA, respectively. R^2
217 was always ≥ 0.98 . Melting curves of the amplicons were generated to ensure that the fluorescence
218 signals originated from specific amplicons and not from primer dimers or other artifacts. This was
219 confirmed by checking the amplification products on 1% agarose gel. Gene copy numbers were
220 calculated with StepOne™ software, version 2.2 (Applied Biosystems). The data were normalised
221 and presented in figures as copies g^{-1} dry soil.

222

223 2.3.3 Race identification of *Fusarium oxysporum* f. sp. *lycopersici* from the experimental field

224 Real time PCR was used to identify Fol from the experimental field. Four primer/probe sets were
225 used to identify the tomato wilt pathogen race, on the basis of rDNA -intergenic spacer and
226 avirulence genes. Real - time PCR trials were conducted using genomic DNA from mycelia isolated
227 from the experimental site and soil DNA. Reaction conditions were established according to the
228 protocol described by Inami et al., (2010).

229

230

231

232 2.3.4 RNA extraction and real-time RT-PCR gene expression analysis

233 In order to study the differential expression of the selected genes in plants pre-planting treated with
234 biocontrol agents and compost, the real time RT-PCR assay was used on RNA samples from roots
235 collected at the end of the tomato crop cycle, which had been conserved at -80°C. The total RNA
236 was extracted by means of an RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) using 0.1 g of
237 tomato roots. The RNA was DNase treated using TURBO DNase (Ambion™, Thermo Fisher
238 Scientific, Waltham, USA), according to the manufacturer's protocol, and then reverse transcribed
239 (500 ng total RNA) using a high capacity cDNA reverse transcription kit (Applied Biosystems).
240 The actin gene was used as a reference gene. The primers for the *LeChi3*, *LeChi9*, *TomB13GluA*,
241 *TomB13GluB*, and *LePr1a* target genes were used according to Aimé et al., (2008). Real-time PCR
242 reactions were carried out with 25 ng of cDNA, 500 nM of each primer, 10 µl of the 1x Power
243 SYBR green master mix (Applied Biosystems) and RNase-free water in a final volume of 20 µl.
244 cDNA was replaced by RNase free water in the negative control. A programme consisting of 15
245 min at 95°C, followed by 40 denaturation cycles for 15 s at 95°C, annealing for 30 s at 58°C and
246 extension for 30 s at 72°C was used for the real-time PCR, at the end of which fluorescence was
247 measured. Real-time PCR reactions were carried out in duplicate for each sample. Primer titration
248 and dissociation experiments were performed to confirm there was no formation of primer dimers
249 or false amplicons that could interfere with the results. After the real-time PCR experiment, the Ct
250 number was extracted for both the reference gene and the target gene considering an auto baseline
251 and a manual threshold. Gene expression levels (relative to the actin gene) were calculated for each
252 cDNA sample using the following equation: $\text{relative gene / actin} = (E_{\text{gene}} - Ct_{\text{gene}}) / (E_{\text{actin}} - Ct_{\text{actin}})$ ratio.
253

254

255 2.4. Chemical properties of the rhizosphere and bulk soil samples

256 The pH values were measured in water suspensions at a solid:liquid ratio of 1:2.5. The total
257 nitrogen (TN) was quantified using a Leco Tru Spect CN automatic analyser. The dissolved organic

258 carbon (DOC) was determined after sample acidification in a TOC/TN analyser (Multi NC 2100S,
259 Analytic Jena GmbH, Jena, Germany). Ammonium (NH_4^+) and nitrate (NO_3^-) were measured
260 colorimetrically, by means of a continuous flow auto-analyser (Alliance Evolution II), using
261 standard colorimetric techniques. The total phosphorus (Pt) was determined by means of 'ICP
262 Varian mod. Liberty LR', after microwave digestion with hydrogen peroxide, hydrochloric acid and
263 nitric acid, filtration and dilution. The available P (P_{av}) was extracted using sodium bicarbonate and
264 determined by means of the molybdenum blue method (Olsen et al., 1954), modified for continuous
265 flow colorimetric analysis (Alliance Evolution II).

266

267 2.5. Statistical analyses

268 The data on gene abundance, on the chemical properties of the soil and on the disease index were
269 subjected to a linear mixed model, with time considered as the random variable, using R software
270 (Software R 3.0.1, R foundation for Statistical Computing, Vienna, Austria, [http://www.R-](http://www.R-project.org)
271 [project.org](http://www.R-project.org)). The effects of two different BCA and compost applications on the abundance of the
272 studied genes, on the chemical properties of the soil and on disease severity were evaluated. All the
273 data were subjected to a Levene test to check for the homogeneity of variance, and normality was
274 tested on the residuals using the Shapiro - Wilk test; when not normally distributed, data from
275 disease severity (DS) were arcsin transformed, while the data from microorganism abundance were
276 log-transformed and normality was checked again. The means were separated by means of the
277 Bonferroni test. The statistical analysis included treatment, year, treatment \times year and rhizosphere \times
278 bulk soil.

279 Pearson's linear correlation coefficients were calculated to assess the relationships between
280 microbial abundance and disease severity as well as between BCA and F_{ol} abundance and the soil
281 chemical properties.

282

283

284 **3. Results**

285 3.1. Disease severity

286 In 2016, in the presence of a low level of disease severity (DS 29.7%) in the untreated control - C
287 tomato plots, the two tested BCAs and composts provided a disease reduction of 89.5 to 93%. The
288 green compost - CV provided statistically similar results to those of the compost - CM, which was
289 enriched with *Trichoderma* sp. TW2 (a disease reduction of 93% and 64%, respectively). However,
290 no significant differences were observed between treatments. In 2017, a disease severity of 39.9%
291 was observed in the untreated tomato control plants, but all the treatments significantly reduced
292 Fusarium wilt symptoms as much as 50%, and statistically similar results between treatments were
293 obtained (Table 3).

294

295 3.2. Microbial abundance

296 *3.2a Race identification and the abundance of soil-borne F. oxysporum f.sp. lycopersici (Fol)*

297 The amplification reaction for the Fol race identification was positive for both the P1 and R1 Race -
298 1 specific sets (Inami et al., 2010), on the basis of the rDNA-IGS and the avirulence genes,
299 respectively (data not shown).

300 In general, Fol abundance was significantly higher in the bulk soil than in the rhizosphere soil, $P(F)$
301 $Rhizo * Bulk\ soil = 0.003$. All the treatments resulted in a significant pathogen reduction, compared
302 to the untreated control - C, in both the rhizosphere and bulk soil at the end of both trials (2016 and
303 2017) (Figure 1). The treatment \times year interaction was not significant.

304

305 *3.2b Total microbial community abundance*

306 At the end of both trials (2016 and 2017), the bacterial, archaeal and fungal communities showed a
307 notable homogeneity, with no significant treatment \times year interaction. Therefore, the data were
308 presented as the average of all the values of both trials.

309 Overall, at the end of both trials, the bacterial 16S rRNA abundance was significantly higher at the
310 bulk soil level than at the rhizosphere level (Table 4). A higher bacterial abundance was observed in
311 rhizosphere soil samples after the *Trichoderma* sp. TW2 - TW2 treatment, than in the untreated
312 control -C, which was significantly similar to the green compost - CV treatment. On the other hand,
313 the highest bacterial abundance at the soil level was observed after the inoculated compost - CM
314 treatment, while the lowest was observed after the *B. subtilis* treatment. The archaeal 16S rRNA
315 gene abundance in the bulk soil samples was more stable and was not influenced by the treatments.
316 However, the green compost - CV, green compost - CM+TW2, and *B. subtilis* treatments resulted in
317 an elevated archaeal presence, compared to the untreated control - C in the rhizosphere soil samples
318 (Table 4). The fungal 18S rRNA abundance was significantly higher in the rhizosphere samples
319 than in the bulk soil ones (Table 4). A significantly higher fungal abundance was observed in the
320 rhizosphere after the inoculated compost - CM, *Trichoderma* sp. TW2 - TW2 treatments as well as
321 in the untreated control - C. *B. subtilis* and green compost CV significantly reduced the total fungal
322 population, while the compost - CM, *Trichoderma* sp. TW2 - TW2 treatments did not affect it. At
323 the bulk soil level, the highest fungal abundance was observed after the green compost - CV
324 treatment, while the untreated control - C was statistically similar to the *Trichoderma* sp. TW2 -
325 TW2 treatment. *B. subtilis* and green compost CM+TW2 significantly reduced the total fungal
326 population.

327

328 3.2c Functional gene abundance

329 In general, the ammonia-oxidising bacterial (AOB) gene abundance was not significantly
330 influenced by the treatments, compared to the untreated control, and was significantly more
331 abundant in the rhizosphere soil than in the bulk soil samples. The highest abundance of AOB was
332 observed in the untreated control - C of the bulk soil and the lowest value was observed for the
333 untreated control - C of the rhizosphere samples (Table 5). In general, the ammonia-oxidising
334 archaeal (AOA) gene abundance was significantly more abundant in the bulk soil samples than in

335 the rhizosphere soil ones (Table 5). The highest abundance in the rhizosphere samples was observed
336 after the compost - CM treatment, while the highest abundance in the bulk soil samples was
337 observed after the compost - CV treatment (Table 5).

338

339 3.2d BCA abundance

340 Overall, at the end of both trials, an increase in the indigenous populations of *Bacillus* spp. and
341 *Trichoderma* spp. was observed for all the treatments, compared to the untreated control - C. No
342 significant treatment \times year interaction was observed. In general, the abundance of *Bacillus* spp.
343 was significantly higher for the *B. subtilis* - SM treatment than for the untreated control - C and for
344 all the other treatments in both the rhizosphere and bulk soil. However, both compost treatments
345 resulted in an increased resident *Bacillus* abundance in the rhizosphere and bulk soils after both
346 trials. Nevertheless, the *Trichoderma* sp. TW2 - TW2 - treatment showed similar abundance to
347 those of the untreated control - C (Table 6).

348 The inoculation with *Trichoderma* sp. significantly increased the overall *Trichoderma* spp.
349 community in both the rhizosphere and in the bulk soils, compared to the untreated control - C. The
350 highest abundance of *Trichoderma* spp. was observed in the rhizosphere after the compost - CM
351 treatment and in the bulk soil after the *Trichoderma* sp. TW2 - TW2 treatment (Table 6). *B. subtilis*
352 - SM resulted in a somewhat lower abundance than the *Trichoderma* sp. - TW2 treatment.

353 In general, the *chiA* gene (*Trichoderma* based chitinase) abundance was significantly influenced by
354 the treatments, compared to the untreated control - C (Table 7). All the treatments at the rhizosphere
355 soil level resulted in significant differences between the *chiA* gene abundances. The abundance of
356 the *chiA* gene was higher in the rhizosphere soil samples for the *Trichoderma* sp. TW2 and the
357 compost - CM treatments and lower and significantly similar to the untreated control - C after the *B.*
358 *subtilis* - SM treatment. All the treatments at the bulk soil level also resulted in a higher *chiA*
359 abundance than the untreated control - C. The highest *chiA* gene abundance at the bulk soil level
360 was observed after the green compost - CV treatment.

361 3.2e Gene expression - analysis with RT-PCR

362 The levels of the transcripts encoding the studied pathogenesis-related (PR) genes were always
363 higher for the presence of Fol on its own (untreated control - C samples) for all the considered
364 genes. The transcript accumulation in the tomato roots differed according to the treatments (Figure
365 2); the transcript level profiles encoding *Chi3* and *Chi9* were rather similar, and were higher in the
366 *Trichoderma* sp. based treatments than the treatments with *B. subtilis* - SM and green compost -
367 CV. Overall, the expression level of *GluA* was lower than the expression level of *GluB*. The *B.*
368 *subtilis* - SM and both compost treatments showed a slightly higher expression of *GluA* than the
369 *Trichoderma* sp. TW2 - TW2 inoculation on its own. The *GluB* gene was expressed more in the *B.*
370 *subtilis* - SM inoculated compost and *Trichoderma* sp. TW2 - TW2 treatments. The expression
371 level of the PR-1 gene was very low for all the treatments.

372

373 3.3. Chemical properties

374 The pH was generally significantly lower ($p < 0.05$) at the rhizosphere level than at the bulk soil
375 level for both trials, with values ranging from 7.43 to 7.60 for the rhizosphere and between 7.95 and
376 8.18 for the bulk soil samples. The concentrations of TN, NH_4^+ , NO_3^- and DOC were generally
377 significantly higher in the rhizosphere samples than in the bulk soil ones. The untreated control - C
378 was characterised by higher NH_4^+ , NO_3^- and DOC concentrations than all the treatments at a bulk
379 level (Table S1). No significant treatment \times year interaction was observed.

380 The dry weight of the roots was not significantly different between the SM, CM and CV treatments.

381 The highest dry weight was observed after the TW2 treatment, while the lowest was observed in the
382 untreated control - C (Figure S1).

383

384 3.5. Correlations between the total microbial communities (16S bacteria, 16S archaea and 18S
385 fungi), the functional genes (fungal *chiA* gene, bacterial and archaeal *amoA* genes.), the BCAs
386 (*Bacillus*, *Trichoderma*) and Fol abundances, and DS.

387 In general, the correlations were negative, with r coefficients ranging from -0.3 to -0.7 ($p < 0.001$)
388 at the rhizosphere level and from -0.3 to -0.8 ($p < 0.001$) at the bulk soil level (Table 8 and Table 9,
389 respectively).

390

391

392 **4. Discussion**

393 In the present study, pre-planting treatments with BCAs (*Bacillus subtilis* and *Trichoderma* sp. -
394 TW2) and two composts were used to control tomato Fusarium wilt caused by *Fusarium oxysporum*
395 f.sp. *lycopersici* (Race 1) in naturally infested soil. The evaluation was based on the two-year effect
396 of these treatments on the disease incidence, PR gene expression and on the rhizosphere/soil
397 microflora and chemical properties. The risk assessment of the BCAs and compost utilised within
398 the frame of this study pointed out the impact these treatments had on the pathogen population and
399 disease control corroborated with the PR gene expression status, as well as on the rhizosphere and
400 bulk soil microbial community dynamics. This impact is important as the resident microbes may
401 colonise similar niches as the newly introduced BCA microorganisms and thus compete for similar
402 resources in the rhizosphere and soil environment (Winding et al., 2004).

403

404 *4.1. Disease severity, and F. oxysporum* f. sp. *lycopersici* (Fol) abundance

405 The preventative application of two BCAs and composts, starting from the nursery, significantly
406 reduced Fol Race 1 abundance, compared to the untreated control. These findings were in accord
407 with the results of previous studies that showed that *Fusarium* abundance at the rhizosphere level of
408 different hosts, such as cucumber, banana and lettuce, was reduced after BCA treatments (Qiu et al.,
409 2012; Shen et al., 2015; Fu et al., 2017). In the present study, Race 1 of Fol abundance was low
410 after the treatment with the enriched compost (CM), thus indicating that an effective Fusarium wilt
411 control may be achieved on tomato with organic amendments, as previously pointed out (Borrero et
412 al., 2004; Cotxarerra et al., 2002). The compost utilisation probably resulted in changes in the

413 activity, density and structure of the non - target microbial community in the soil, thus enhancing
414 competition with the pathogen for space and nutrients, such as carbon (C) and nitrogen (N). This
415 may have resulted in a reduction in the Fol abundance and its potential activity, as already reported
416 by Bonilla et al., (2012), Cucu et al., (2018), Larkin et al., (2015).

417

418 4.2. Plant response to *Fol* infection

419 Plants usually have a complex defence system against pathogens that are well known as stress
420 factors, including different types of stress proteins with putative protective functions (Brown et al.,
421 2017). A high accumulation of transcripts encoding the studied PR genes in the plant roots from the
422 untreated control was observed in this study. This may be correlated to the colonisation of the
423 tomato vessels by Fol, as previously reported by Olivain and Alabouvette, (1999). The here
424 presented results are in line with those of Aime' et al., (2008), who found a higher PR gene
425 expression in tomato plants inoculated with a pathogenic strain, than in plants inoculated with a
426 non-pathogenic strain of *F. oxysporum*. Moreover, the pre-inoculation of plants with *Trichoderma*
427 sp. resulted in an even higher expression of PR genes encoding chitinase, thus indicating that
428 *Trichoderma* spp. induced a potentiated status in the plant that enabled it to be more resistant to
429 subsequent pathogen infection, as was also observed by Shores et al., (2005). This hypothesis is in
430 line with that of Shores et al., 2005 and Yedidia et al., 2003, who found that, in a *T. asperellum* -
431 cucumber system, PR protein chitinase, β -1,3-glucanase and peroxidase were induced by
432 *Trichoderma*. Jangir et al., (2018) showed that *Bacillus* sp. produced chitinase and β -1, 3-glucanase
433 that might play a role in the digestion of the pathogen's hyphae as carbon used than as energy
434 sources. Bargabus et al., (2002) showed that the treatment of sugar beet with *Bacillus mycoides*
435 induced the expression of PR genes that is new isoforms of β -1,3-glucanase. The potential
436 production of PR-proteins with chitinase and β -1,3- glucanase activity is presumably part of the
437 hypersensitive defence mechanism of tomato plants and may be responsible for the induction of the
438 resistance developed by the plant after treatment with the antagonists and infection with Fol.

439 4.3. Ecological feedback of the applied treatments and their effect on the total indigenous
440 prokaryotes

441 Rhizosphere microbial communities play important roles in plant health and disease prevention
442 (Bonanomi et al., 2018; Dudenhöffer et al., 2016). Analysing the abundance of the BCA - like
443 microorganisms, introduced into the soil as strains or through the compost treatments, showed a
444 very good feedback of the targeted populations (e.g., *Bacillus* spp. and *Trichoderma* spp.) as well as
445 excellent efficiency, as highlighted by the strong negative correlations with disease severity.

446 The abundance of *Trichoderma* spp. showed different trends in all the treatments. A higher
447 abundance of *Trichoderma* was detected in the rhizosphere of the inoculated compost treatment, but
448 also after the inoculation of the single strain. It is well known that composts represent an optimal
449 substrate for *Trichoderma* spp., as the organic matter composition and the associated biotic and
450 abiotic conditions can in general influence *Trichoderma* activity (Vinale et al., 2008; Lorito and
451 Woo, 2015). *Trichoderma* agents have been reported to be antagonistic to the *Fusarium* pathogens:
452 *F. oxysporum* f. sp. *ciceris*, *F. solani*, *F. oxysporum* f. sp. *lycopersici* (Dubey et al., 2007; Rojo et
453 al., 2007; Christopher et al., 2010). Gachomo and Kotchoni (2008) revealed that *Trichoderma*
454 isolates displayed various extracellular enzyme activities, for example amylase, chitinase, pectinase,
455 protease, lipase and cellulase activities, in order to compete with other microbes, and that the
456 production of volatile compounds by *Trichoderma* species could inhibit the growth of pathogenic
457 microorganisms. One of the main mechanisms of *Trichoderma* in the control of the soil-borne
458 pathogen is due to the presence of genes encoding cell wall degrading enzymes (Lorito et al., 1998,
459 Lorito and Woo, 2015). In this regard, the abundance of the *chiA* gene and its negative correlation
460 with disease severity has confirmed the potential antagonistic activity of the inoculated
461 *Trichoderma* strain TW2, but also of the inoculated compost - CM. This result has pointed out an
462 enhanced nitrogen efficiency as a result of the improvement of the nitrogen reduction and
463 assimilation mechanism, as also evidenced by Lorito et al., (2010) and Borrero et al., (2012). In
464 addition, the competition for carbon and other growing factors at the rhizosphere level helped to

465 sustain the control of Fol by *Trichoderma*, as highlighted by the very good correlation with
466 dissolved organic carbon at the rhizosphere and bulk soil levels as well as with the ammonium
467 content at the rhizosphere level. These results support the results of Sivan and Chet (1989), who
468 demonstrated that the competition for nutrients is the major mechanism used by *T. harzianum* to
469 control *F. oxysporum* f. sp. *melonis*.

470 The *Bacillus* sp. treatment also resulted to be effective in reducing the disease. The abundance
471 results highlighted the excellent feedback of *Bacillus* spp. from the rhizosphere and soil levels. The
472 significant negative relationships ($p < 0.001$) between *Bacillus* spp. and disease severity pointed out
473 the antagonistic function of *Bacillus* against Fol, which may be due to volatile compounds and
474 siderofori production, as reported by Jangir et al., (2018) after having characterised antagonistic
475 *Bacillus* sp., isolated from a tomato rhizosphere, and to its control mechanisms against Fol.

476 Although a significant suppression of Fusarium wilt was achieved when the BCAs were employed
477 individually (i.e., SM, TW2), alone or together with compost (i.e., CM), it is possible to assume, in
478 agreement with Baker (1990), that the effects that were observed on antagonistic microbial
479 communities after BCA applications may be the key to a successful and integrated approach for the
480 control of tomato Fusarium wilt. In addition, as reported by Vinale et al., (2008), the biotic
481 components of the soil environment also have relevant effects on the activity of the used biocontrol
482 agents.

483 Overall, the release and successful proliferation of BCAs in the studied soil did not negatively alter
484 the abundance of the functionally relevant indigenous soil microorganisms. The here presented
485 results support previous reports which were based on the analysis of microbial densities (Ghini et
486 al., 2000; Gullino et al., 1995; Mezzalama et al., 1998). A higher total fungal abundance was
487 observed in the rhizosphere than in the bulk soil. The increased fungal community that was
488 observed for all the treatments and for the untreated control was probably caused by the elevated
489 level of organic carbon (Table S1). This may be linked to a competitive potential, based on an
490 increased root exudation, which favours rhizosphere colonisation by fungi, including Fol and

491 *Trichoderma* spp. This result has been corroborated by considering the root dry weight (Figure S1).
492 According to Griffiths and Philippot (2013), the results suggested a great tolerance and resilience
493 potential of the native fungal community towards invading microorganisms (BCAs). Similarly,
494 Edel-Hermann et al., (2009) and Savazzini et al., (2009) only observed transient community shifts
495 in indigenous microbial populations in response to inoculation with antagonistic *Fusarium*
496 *oxysporum* and *Trichoderma atroviride*. However, a difference among treatments was observed at
497 both rhizosphere and bulk soil level, as *B. subtilis* and compost resulted in a decrease of total fungal
498 abundance. This result highlighted on one hand the rhizosphere competence of *B. subtilis* which
499 probably had a quick take over of space and nutrients. On the other hand the capacity of plant to
500 select the beneficial microorganisms as well as the influence of soil characteristics are well known
501 to be keys of soil biomass density and composition (Chapparo et al., 2012) Compost and
502 formulations of compost with bacteria or fungi (e.g., *Trichoderma* W2) may also dictate
503 competition for nutrients and the predominance of some microbial groups to another (Pugliese et al.
504 2011).

505 A high resilience of the total archaeal community was observed at both the rhizosphere and the soil
506 level, compared to the total bacterial community. Bacterial abundance was lower at the rhizosphere
507 level than at the bulk soil level, probably as a result of increased competition with the fungal
508 counterparts, including Fol. Archaeal nitrifiers (AOA) were more abundant in the bulk soil samples
509 than in the rhizosphere samples, which may be a result of the lower organic carbon content of the
510 soil and the higher pH (Wessén et al., 2010; Bates et al., 2011). A negative correlation was found
511 between the abundance of AOA and Race 1 of Fol at a soil level, thus suggesting a direct effect of
512 archaeal nitrifiers on the pathogen. However, these speculative interpretations require further
513 research to clarify the mode of actions of AOA responses to the development of Fol in the soil of
514 tomatoes. Nevertheless, the higher AOA abundance in the soil samples indicated that the AOA had
515 probably adapted to the decomposition of recalcitrant organic matter, as already described by Cucu
516 et al. (2017). The same pattern was observed by Cucu et al., (2018) for a lettuce - *Fusarium* wilt

517 pathosystem. This highlights the importance of abiotic factors, such as pH and soil type, in driving
518 not only the dynamics of prokaryotes, but also a higher level of suppressiveness towards *Fusarium*
519 spp. in the soil environment with pH ~/8. This finding supports the findings of earlier studies which
520 correlated soil suppressiveness to *Fusarium* spp. with such abiotic soil characteristics as the clay
521 content and pH (Höper et al., 1995; Yergeau et al., 2010). The bacterial nitrifiers showed a higher
522 abundance and, as a consequence, a higher resilience at the rhizosphere level than the archaeal
523 counterparts. This result, as already pointed out by Wessén et al., (2010), points out that a low
524 ammonia environment is the key factor that determines a niche separation of AOA and AOB in
525 neutral soils.

526 The effect of *Bacillus* sp. and *Trichoderma* sp. on the nitrifiers was similar between treatments, thus
527 suggesting that *Bacillus subtilis* and *Trichoderma* sp. TW2 may not have any negative ecological
528 impact on other groups of microorganisms

529

530 **Conclusions and outlook**

531 The results have pointed out that the application of *Bacillus subtilis*, *Trichoderma* sp. TW2 and
532 compost treatments is safe for the rhizosphere and soil resident non-target microbial communities as
533 well as for the effective control of Fol on tomato. Moreover, the different response of the AOA
534 population to various treatments at a rhizosphere and a bulk level could indicate their suitability as
535 indicators to assess perturbations in soils, for example as the result of the introduction of new
536 microorganisms which act as biocontrol agents in the soil environment. Nevertheless, for a better
537 understanding of the impact that different treatments (e.g., biological or chemical) may have on
538 *Fusarium oxysporum* f.sp. *lycopersici*, it is necessary to consider not only non-target soil microbial
539 communities, but also different soil types characterised by different physical - chemical properties,
540 as abiotic factors.

541

542

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547

548

549 **Conflict of Interest**

550 The authors declare that they have no conflict of interest. Massimo Pugliese declares he has a
551 financial interest (shareholder) in the AgriNewTech company that provided some of the products
552 tested in this study.

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798 **Figure Captions**

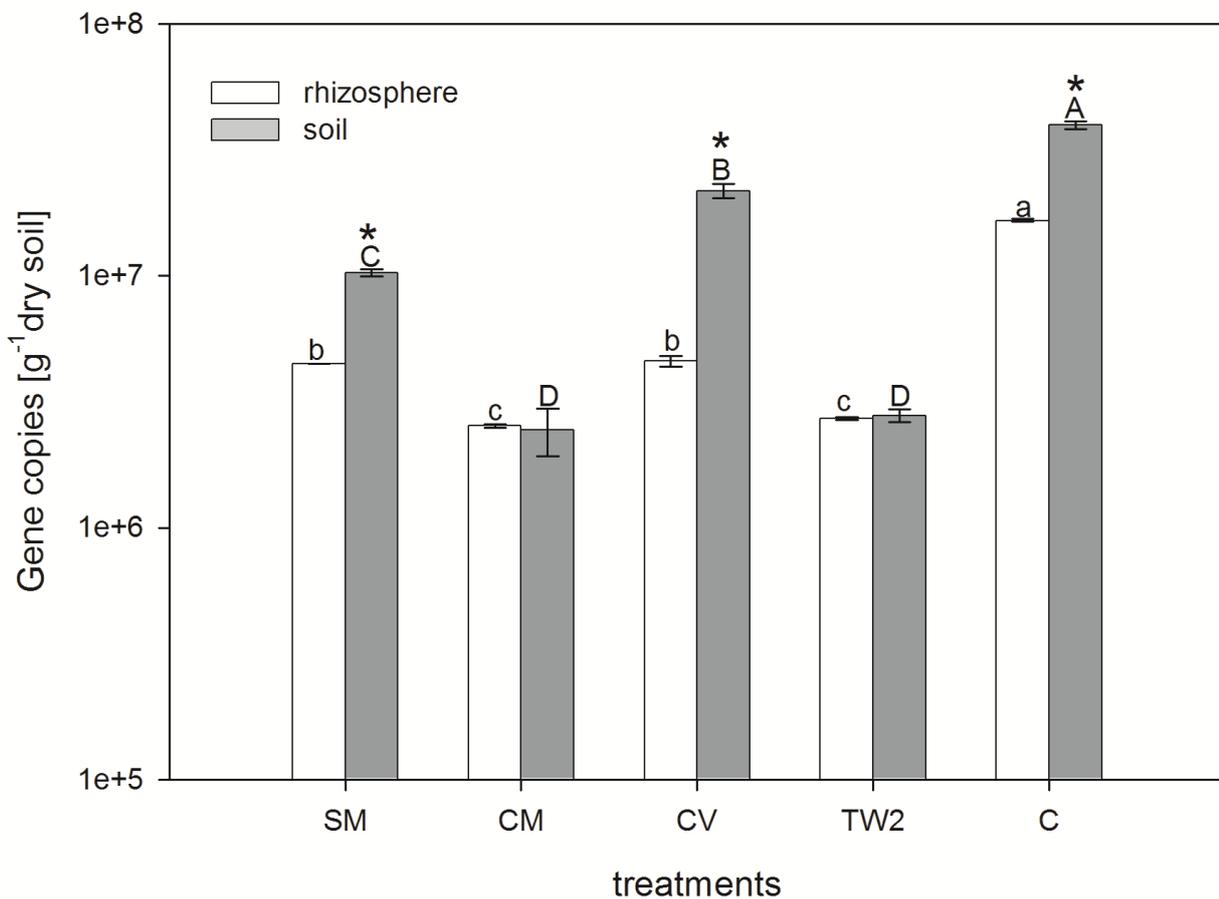
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800 **Figure 1** Abundance of *Fusarium oxysporum* f.sp. *lycopersici* (Fol) in the rhizosphere and bulk soil
801 for different treatments and the untreated control (SM - *Bacillus subtilis* - Serenade Max; CM -
802 Green compost plus *Trichoderma* sp. TW2; CV - green compost; TW2 - *Trichoderma* sp. TW2; C -
803 untreated control) (n = 6, means±standard errors). Different letters above the bars indicate
804 significant differences between treatments in the rhizosphere (uppercase letters) and bulk soil
805 (lowercase letters); *indicate significant differences between rhizosphere and bulk soil

806 **Figure 2** Real-time RT-PCR analysis of PR gene expression in the tomato roots for different
807 treatments and for the untreated control. Actin was used as the reference gene. The error bars show
808 standard deviations for triplicate assays.

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Fusarium oxysporum f.sp. *lycopersici*



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812 Figure 1

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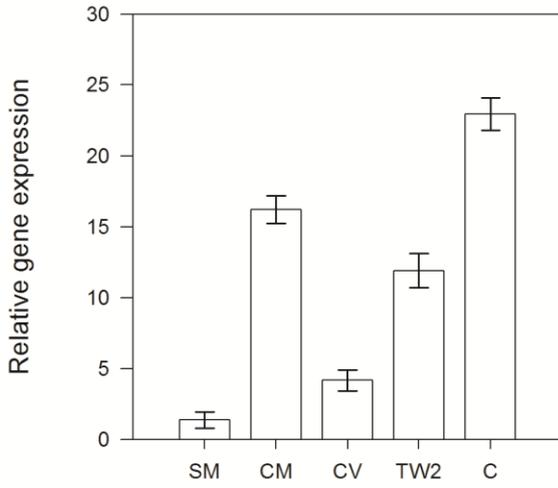
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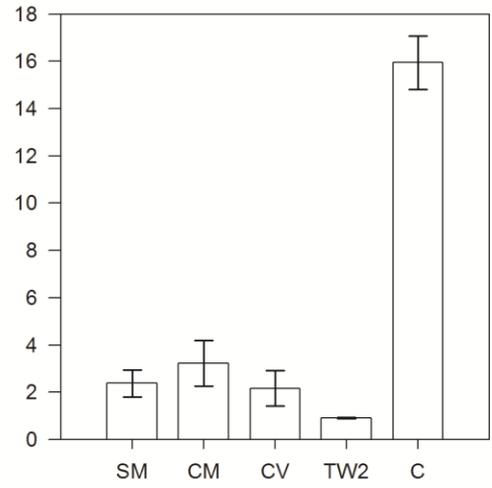
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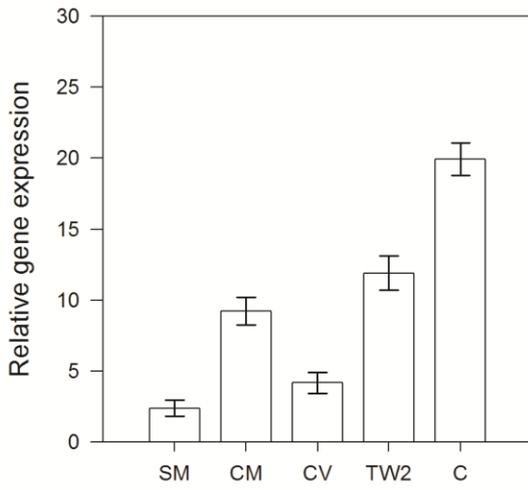
chi3



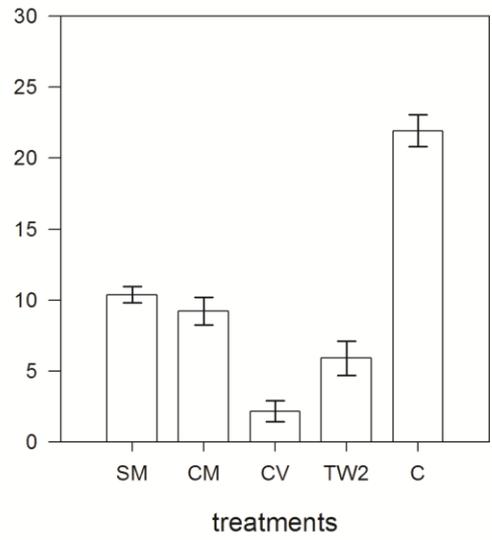
GluA



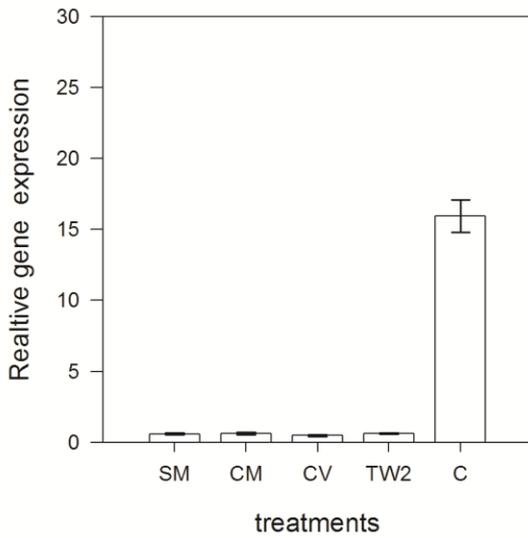
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823 Figure 2

824 Tables

825 Table 1 General information on the trials and timing of the operations carried out in 2016 and 2017

Treatment	Microorganism/a.i.	Dosage	Tray treatment	Plot Treatment
Serenade max - SM	<i>Bacillus subtilis</i> QST 713	2.9X10 ¹⁰ cells/L water	T37; T44; T51;T58*	-
ANT'S COMPOST M - CM	<i>Green compost + Trichoderma sp. TW2</i>	8 g/seedling;1kg/0.1m ³ of soil	T0	- T51
ANT'S COMPOST V - CV	<i>Green compost</i>	8 g/seedling; 1kg/0.1m ³ of soil	T0	- T51
<i>Trichoderma sp. TW2 -TW2</i>	<i>Trichoderma sp. TW2</i>	1x10 ⁷ (conidia /ml)	T37; T44; T51;T58	
Untreated control - C	-	-		

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827 *Treatments: T0 at sowing; T1, 37 days after sowing; T2, 44 days after sowing; T3: 51 days after sowing; T 4: 58 days after sowing and immediately
828 before transplanting.

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845 Table 2 Description of the primer sets and amplification details used for the quantitative PCR.

Target group	Primer (reference)	Amplification details
<i>Fusarium oxysporum</i> f.sp. <i>lycopersici</i> (Fol)	FO1(Prashant et al., 2003) 5'-ACATACCACTTGTTGCCTCG-3'	40 cycles 95°C 15s, 60°C 60s, 72°C 45s
	FO2 (Prashant et al., 2003) 5'-CGCCAATCAATTTGAGGAACG-3'	
All bacteria (16S rRNA gene)	Eub338 (Lane 1991) 5'-ACTCCTACGGGAGGCAGCAGCAG-3'	40 cycles 95°C 30s, 55°C 35s, 72°C 45s
	Eub518 (Muyzer et al. 1993) 5'-ATTACCGCGGCTGCTGG-3'	
All archaea (16S rRNA gene)	340F (Gantner et al., 2011) 5'-CCCTAYGGGGYGCASCAG-3'	40 cycles 95°C 30s, 57°C 30s, 72°C 30s
	1000R (Gantner et al., 2011) 5'-GGCCATGCACYWCYTCTC-3'	
All fungi (18S rRNA gene)	FR1 (Vainio and Hantula, 2000) 5'-AIC CAT TCA ATC GGT AIT-3'	45 cycles 95°C 30s, 50°C 30s, 70°C 60s
	390FF (Vainio and Hantula, 2000) 5'-CGA TAA CGA ACG AGA CCT-3'	
Ammonia oxidizing bacteria (AOB)	AmoA-1f (Rotthauwe et al. 1997) 5'-GGGGTTTCTACTGGTGGT-3'	45 cycles 95°C 30s, 57°C 45s, 72°C 45s, 78°C 20s
	AmoA-2r (Rotthauwe et al. 1997) 5'-CCCCTCKGSAAAGCCTTCTTC-3'	
Ammonia oxidizing archaea (AOA)	Arch-amoAf (Francis et al., 2005) 5'-STAATGGTCTGGCTTAGACG-3'	45cycles 95°C 30s, 53°C 45s, 72°C 45s, 78°C 20s
	Arch-amoAr (Francis et al.,2005) 5'-GCGGCCATCCATCTGTATGT-3'	
<i>chiA</i> gene	chiaxf (Cucu et al., 2018) 5'-ACCCTGCCGATGACTACTCAG-3'	35cycles 95°C 15s, 59.9°C 30s, 70°C 30s
	chiaxr (Cucu et al., 2018) 5'-GGCAGCGATGGAGAGAAGGA-3'	
<i>Bacillus subtilis</i>	Forward <i>B. subtilis</i> (Gao et al., 2011) 5'-TCGCGGTTTCGCTGCCCTTT-3'	40 cycles 95°C 30s, 60°C 60s, 80°C 10s
	Reverse <i>B. subtilis</i> (Gao et al., 2011) 5'-AAGTCCCGCAACGAGCGCAA-3'	
<i>Trichoderma</i> spp.	uTf (Hagn et al., 2007) 5'-AACGTTACCAAACCTGTTG-3'	35 cycles 95 °C 30 s, 55.5 °C 30 s, 72 °C 30 s
	uTr (Hagn et al., 2007) 5'-AAGTTCAGCGGTATTCT-3'	

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852 Table 3 Effect of the preventative soil treatments with BCAs and compost on disease severity at the
 853 end of trials 1 and 2, in 2016 and 2017, respectively

Treatment	Disease severity**			
	%			
	2016		2017	
<i>Bacillus subtilis</i> QST 713 - SM	6.3	±3.8 ^a	19.5	±3.0 ^a
<i>Green compost + Trichoderma</i> sp. TW2 - CM	10.4	±5.2 ^a	21.9	±2.8 ^a
<i>Green compost</i> - CV	1.9	±1.9 ^a	14.5	±2.6 ^a
<i>Trichoderma</i> sp. TW2 - TW2	3.1	±3.1 ^a	16.0	±1.3 ^a
Untreated control - C	29.7	±2.3 ^b	39.9	±2.4 ^b

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*Treatments: T0 at sowing; T1, 37 days after sowing; T2, 44 days after sowing; T3: 51 days after sowing; T 4: 58 days after sowing and immediately before transplanting.

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**Sixteen plants/treatment were evaluated for disease severity using the following rating scale: 0 = healthy plant, 25 = initial leaf chlorosis, 50 = severe leaf chlorosis and initial symptoms of wilting during the hottest hours of the day, 75 = severe wilting and severe symptoms of leaf chlorosis; 100 = plant totally wilted, leaves completely necrotic.

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883 Table 4 Abundance of 16SrRNA (all bacteria and archaea) and 18S rRNA (fungi) in the rhizosphere
 884 and bulk soil after different treatments and in the untreated control

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Treatment	Rhizosphere soil	Bulk soil
<i>16S Bacteria (logcopy DNA⁻¹)</i>		
<i>Bacillus subtilis</i> (SM)	10.686 c	10.963 d
Green compost+TW2 (CM)	10.755 b	11.287 a
Green compost (CV)	10.454 d	10.904 d
<i>Trichoderma</i> sp. TW2 (TW2)	10.903 a	11.191 b
Untreated control - C	10.477 d	11.077 c
<i>P(F) Treat</i>	0.000	
<i>P (F) Year</i>	0.000	
<i>P(F) Treat*Year</i>	ns	
<i>P(F) Rhizo*Bulk soil</i>	0.000	
<i>16S archaea (logcopy DNA⁻¹)</i>		
<i>Bacillus subtilis</i> (SM)	7.728 a	7.995 a
Green compost+TW2 (CM)	7.748 a	7.982 a
Green compost (CV)	7.751 a	8.023 a
<i>Trichoderma</i> sp. TW2 (TW2)	7.592 b	7.974 a
Untreated control - C	7.549 b	7.988 a
<i>P(F) Treat</i>	0.000	
<i>P (F) Year</i>	0.000	
<i>P(F) Treat*Year</i>	ns	
<i>P(F) Rhizo*Bulk soil</i>	0.002	
<i>18S fungi (logcopy DNA⁻¹)</i>		
<i>Bacillus subtilis</i> (SM)	9.669 c	8.955 b
Green compost+TW2 (CM)	10.111 a	8.927 b
Green compost (CV)	9.813 b	9.226 a
<i>Trichoderma</i> sp. TW2 (TW2)	10.000 a	8.631 c
Untreated control - C	9.979 a	8.542 c
<i>P(F) Treat</i>	0.000	
<i>P (F) Year</i>	0.000	
<i>P(F) Treat*Year</i>	ns	
<i>P(F) Rhizo*Bulk soil</i>	0.000	

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888 Table 5 Abundance of bacterial (AOB) and archaeal (AOA) *amoA* genes in the rhizosphere and
 889 bulk soil after different treatments and in the untreated control

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Treatment	Rhizosphere soil		Bulk soil	
	<i>AOB (logcopy DNA⁻¹)</i>			
<i>Bacillus subtilis</i> (SM)	7.316	a	6.233	c
Green compost+TW2 (CM)	7.280	a	6.665	b
Green compost (CV)	7.321	a	6.686	b
<i>Trichoderma</i> sp. TW2 (TW2)	7.387	a	6.603	b
Untreated control - C	7.125	b	6.877	a
<i>P(F) Treat</i>	0.000			
<i>P(F) Year</i>	0.000			
<i>P(F) Treat*Year</i>	ns			
<i>P(F) Rhizo*Bulk soil</i>	0.007			
	<i>AOA (logcopy DNA⁻¹)</i>			
<i>Bacillus subtilis</i> (SM)	6.657	b	7.915	b
Green compost+TW2 (CM)	6.974	a	7.884	b
Green compost (CV)	6.721	b	8.149	a
<i>Trichoderma</i> sp. TW2 (TW2)	6.674	b	7.935	b
Untreated control - C	6.681	b	7.926	b
<i>P(F) Treat</i>	0.000			
<i>P(F) Year</i>	0.000			
<i>P(F) Treat*Year</i>	0.015			
<i>P(F) Rhizo*Bulk soil</i>	0.000			

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903 Table 6 Abundance of *Bacillus subtilis* and *Trichoderma* spp. genes in the rhizosphere and bulk soil
 904 after different treatments and the untreated control

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Treatment	Rhizosphere soil		Bulk soil	
<i>Bacillus (logcopy DNA⁻¹)</i>				
<i>Bacillus subtilis</i> (SM)	6.024	a	6.458	a
Green compost +TW2 (CM)	4.702	c	5.798	b
Green compost (CV)	5.980	b	5.662	c
<i>Trichoderma</i> sp. TW2 (TW2)	4.673	d	4.865	d
Untreated control - C	4.685	d	4.931	d
<i>P(F) Treat</i>	0.000			
<i>P (F) Year</i>	0.000			
<i>P(F) Treat*Year</i>	ns			
<i>P(F) Rhizo*Bulk soil</i>	0.013			
<i>Trichoderma (logcopy DNA⁻¹)</i>				
<i>Bacillus subtilis</i> (SM)	4.762	d	3.731	c
Green compost+TW2 (CM)	6.922	a	4.483	a
Green compost (CV)	5.494	c	3.652	b
<i>Trichoderma</i> sp. TW2 (TW2)	6.832	b	4.632	a
Untreated control - C	4.791	d	3.977	c
<i>P(F) Treat</i>	0.000			
<i>P (F) Year</i>	0.000			
<i>P(F) Treat*Year</i>	ns			
<i>P(F) Rhizo*Bulk soil</i>	0.016			

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916 Table 7 Abundance of the *chiaA* gene in the rhizosphere and bulk soil after different treatments and
 917 the untreated control

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Treatment	Rhizosphere soil		Bulk soil	
	<i>chiA</i> gene (logcopy DNA ⁻¹)			
<i>Bacillus subtilis</i> (SM)	3.632	c	4.384	b
Green compost+TW2 (CM)	5.736	a	4.363	b
Green compost (CV)	4.338	b	4.403	a
<i>Trichoderma</i> sp. TW2 (TW2)	5.789	a	4.321	b
Untreated control - C	3.373	c	3.890	c
<i>P(F) Treat</i>	0.000			
<i>P (F) Year</i>	0.000			
<i>P(F) Treat*Year</i>	ns			
<i>P(F) Rhizo*Bulk soil</i>	0.015			

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922 Table 8 Pearson's correlation coefficient for the BCAs, Fol race 1 abundance and chemical
 923 properties in the rhizosphere (rhizo) and bulk soil samples
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Property	<i>Bacillus subtilis</i>		<i>Trichoderma</i> spp.		Fol	
	rhizo	bulk soil	rhizo	bulk soil	rhizo	bulk soil
pH	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
TN	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
NH ₄ ⁺	<i>ns</i>	<i>ns</i>	0.78***	<i>ns</i>	<i>ns</i>	<i>ns</i>
NO ₃ ⁻	-0.5	<i>ns</i>	-0.75***	<i>ns</i>	<i>ns</i>	<i>ns</i>
TP	-0.60***	-0.40**	0.5**	<i>ns</i>	-0.4**	-0.5**
AP	0.40**	-0.50**	0.67**	0.50**	0.575**	0.468*
DOC	-0.7***	0.5 *	-0.8***	0.67***	0.676***	0.67**

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 927 Significance levels: not significant-ns: p>0.05; *p<0.05; **p<0.01; ***p<0.001

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939 Table 9 Pearson's correlation coefficients between microbial gene abundance and disease severity
940 (DS) in rhizosphere (rhizo) and bulk soil samples

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960 Significance levels: not significant-ns: $p > 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

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Microbial abundance	Disease severity (DS)	
	rhizo	bulk soil
Fol	0.75***	0.63***
16S Bacteria	ns	ns
16S Archaea	ns	ns
18S Fungi	0.67	ns
AOB	-0.55**	0.65***
AOA	-0.57***	-0.76***
<i>Bacillus</i>	-0.45**	-0.47*
<i>Trichoderma</i>	-0.75***	-0.60***
<i>chiA</i> gene	-0.55***	-0.65***