IrIS AperTO

UNIVERSITÀ DEGLI STUDI DI TORINO

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

**Effects of biocontrol agents and compost against the Phytophthora capsici of zucchini and their impact on the rhizosphere microbiota**

**This is a pre print version of the following article:**

*Original Citation:*

*Availability:*

This version is available http://hdl.handle.net/2318/1762465 since 2020-11-11T17:33:29Z

*Published version:*

DOI:10.1016/j.apsoil.2020.103659

*Terms of use:*

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

05 August 2024

1 **Effects of biocontrol agents and compost against *Phytophthora capsici* of zucchini and the**
2 **impact on the rhizosphere microbiota**
3
4 **Maria Alexandra Cucu**[a,b], Giovanna Gilardi[b], Massimo Pugliese[a,b,c], Ilario Ferrocino[b], Maria
5 Lodovica Gullino[a,b]
6
7 [a]*AGROINNOVA - Centre of Competence for the Innovation in the Agro-Environmental Sector,*
8 *Turin University, Largo P. Braccini 2, 10095, Grugliasco, Turin, Italy*
9 [b]*Agricultural, Forestry and Food Sciences Department (DISAFA), Turin University, Largo P.*
10 *Braccini 2, 10095 Grugliasco, Turin, Italy*
11 [c]*AgriNewTech srl, Via Livorno 60, 10144 Turin, Italy.*
12
13
14
15 *Corresponding author:
16 **Dr. Maria Alexandra Cucu**
17 Phone: +39 011 670 8544
18 Email: mariaalexandra.cucu@unito.it
19
20
21
22
23
24
25
26

27 *Abstract*

28

29 Four different biocontrol agents (BCA’s) and two composts have been used in this study to evaluate
30 their efficiency against Phytophthora blight of zucchini caused by *Phytophthora capsici*. The effects
31 of the BCA’s and composts on the zucchini rhizosphere’s microbiota were examined at the end of
32 experimental trials from 2016 and 2017 in two farms under natural and artificially infested soils.,
33 Next generation amplicon sequencing technology and quantitative Polymerase Chain Reaction -
34 qPCR as targeted and untargeted approaches respectively, have been used. The abundance of *P.*
35 *capsici,* measured for all the treatments over both years, decreased with respect to the untreated
36 control, and it may be assumed that direct pathogen-beneficial microorganism interaction occurred.
37 All treatments reduced the disease incidence by at least 50% in both sites under naturally or
38 artificially infested soil and at both sampling times. The disease index was in accordance with the
39 pathogen abundance. The development of microbiota and mycobiota was not affected by the
40 BCA’s, thus indicating that the tested treatments did not interact negatively with microbial
41 communities, which in turn suggested a slow but good adaptation of the biocontrol agents to the
42 environment. The combination of targeted and untargeted approaches may help to understand the
43 effect of different BCA’s on the development of *Phytophthora capsici*.

44

45

46 **Keywords:** *Phythopthora capsici*; BCA’s; disease suppression; Next Generation Sequencing

47

48

49

50

51

52

53 1. Introduction

54 Zucchini (*Cucurbita pepo* L.) is a very important crop from economically point of view (Jeffrey
55 2001; Paris and Maynard, 2008). The covered growing area in northern Italy is approximately 5,473
56 ha, with 1,326,811 tons of total production in open fields and under greenhouse conditions (ISTAT,
57 2017). This crop is often subjected to attacks by several soil-borne pathogens, one of which,
58 *Phytophthora capsici* (Leon.), is a very serious agent of crown and root rot of cucurbit as well as of
59 other crops (Hausbeck and Lamour, 2004; Lamour et al., 2012). Symptoms caused by *P. capsici* on
60 zucchini have been observed extensively throughout Italy and are very serious in open fields
61 (Garibaldi and Gullino, 2010).

62 The ability to develop fungicide resistance (Hausbeck and Lamour, 2004; Ploetz et al., 2002;
63 Tamietti and Valentino, 2001), the existence of several physiological races and the lack of resistant
64 cultivars (Sy et al., 2008; da Costa Ribeiro and Bosland, 2012), as well as the decreasing
65 availability of fumigants (Colla et al., 2012) are making the disease control very difficult. Thus,
66 there is a need for evolving environmentally friendly stratagems (Parra and Ristaino, 2001) such as
67 the biological control. *Trichoderma* genus is generally known to have a good biocontrol activity
68 (Howell, 2003; Harman 2000; Harman et al., 2004; Verma et al., 2007; Lorito et al., 2010; Lorito
69 and Woo, 2015a; Woo et al., 2014). Antagonistic isolates of *Trichoderma* spp. have in fact been
70 screened for their ability to control chili blight caused by *P. capsici* (Vinale et al., 2008; Lombardi
71 et al., 2018). For example, *T. harzianum* and *T. asperellum* have resulted to be effective against *P.*
72 *capsici* in different pathosystems (Ezziyyani et al., 2007; Segarra et al., 2007, 2013; Jiang et al.,
73 2016). *Bacillus* genus have also shown the ability to reduce the presence of a variety of soil-borne
74 phytopathogenic fungi and oomycetes, including *P. capsici* (Lee et al., 2008; Zhang et al., 2010).
75 Also other microorganisms have been assessed for potentially control *P. capsici*, including
76 *Streptomyces* spp. (Sang et al., 2008; Nguyen et al., 2012), *Paenibacillus* spp. (Kim et al., 2009,
77 Naing et al., 2014), *Clitocybe nuda* (Chen et al., 2012) and *Aspergillus* spp. (Kang and Kim, 2004).
78 In addition, organic amendments, such as composts, have shown a suppressive activity and positive

79 effect on the management of Phytophthora root and crown rot of bell pepper (Chae et al., 2006;
80 Gilardi et al., 2013; Kim et al., 1997; Nunez-Zofio et al., 2011; Sang et al., 2010).
81 Biological control is often based on the rhizosphere competence which includes soil environment
82 colonization of biocontrol agents (BCA's) alone, or mixed with composts (Garibaldi et al., 1989;
83 Gupta et al., 2014; Lorito and Woo, 2015b; Philippot et al., 2013; Wallenstein, 2017; Bonanomi et
84 al., 2018). Since rhizosphere represents the pivotal interface for plant roots and the beneficial
85 microorganisms which have a very import role in plant health, understanding how BCA's and
86 compost applications influence them under field conditions over several seasons of monoculture,
87 may be helpful for the development of practices to suppress pathogens and for the success and
88 fitness of the crop in the agro system.
89 In this study, the effectiveness of BCA's commercially available (*Bacillus subtilis* QST713 and
90 *Trichoderma gamsii* ICC 012 + *Trichoderma asperellum* ICC 080) and experimental (*Trichoderma*
91 *sp*. TW2 and a mixture *of Trichoderma sp. FC7* and *FC8*), together with compost, has been
92 evaluated under field conditions and over two years, in naturally and artificially infested soil
93 conditions. The following objectives were proposed: i) the estimation of the pathogen and disease
94 reduction following the use of BCA's and compost as treatments applied in pre-planting stage; ii)
95 the evaluation of the extent of rhizosphere colonization by BCAs; iii) the evaluation of BCA's and
96 compost impact may have on the rhizosphere microbiota.
97
98 ## 2. Materials and Methods
99 ### 2.1. Layout of the field experiments
100 The experiments were carried out under field conditions in Northern Italy in one experimental farm
101 at Carmagnola (44°88'55.188"N 7°68'37.457"E) as well as in a commercial farm, at Moretta
102 (44°45'49.75"N 7°32'29.18"E). The soils in both sites are silty loam mixed, mesic, Entisoil Typic
103 Ustifluvent (Carmagnola) and Entisoil Aquic Udifluvent (Moretta) (Soil Survey Staff, 2010). The
104 principal difference between the sites is their pH, which is almost neutral for Moretta (pH = 6.85)

105 and alkaline (pH = 7.89) for Carmagnola. The studied soil sites are characterized by silt:sand:clay in
106 a ratio of 60:30:10% and 1.5% organic matter at Moretta, with *Phythopthora capsici* naturally
107 infested soil, and by silt:sand:clay 64:24:12% and 1.5% organic matter at Carmagnola, artificially
108 infested with a selected pathogenic isolate of *P. capsici* obtained from the crown tissues of affected
109 zucchini plants (100 g pathogen biomass per square meter).
110 In both trials carried out in the experimental farm from Carmagnola, a highly virulent strain of *P.*
111 *capsici* (PHC1/16) obtained from infected zucchini plants in a field in Northern-Italy was used. The
112 isolate was propagated by inoculation of a colonized agar–plug on to a sterile mixture of wheat-
113 hemp kernels (2:1 v/v) in a 1-L flask kept at room temperature in the dark (Gilardi et al., 2016). The
114 15-day-old culture of the pathogen was mixed into the soil before planting the treated and untreated
115 seedlings. In order to achieve a uniform soil infestation, the pathogen biomass obtained was
116 incorporated in the soil 7 days before planting at 100 g/m$^2$ by rototilling at a depth of 1–20 cm. Two
117 trials were also carried out in the Moretta commercial farm, naturally infested with the pathogen.
118 The site of the trials was selected because had a history of zucchini cultivation with 20 to 40% of
119 plant losses depending the years.
120 The field trials were conducted over two consecutive years, that is, 2016 and 2017 in order to
121 evaluate the efficacy of soil treatments applied in pre-planting starting from the nursery against *P.*
122 *capsici* with different biocontrol agents (BCA's) such as *Bacillus subtilis* QST713 (Serenade, Bayer
123 Crop Science, Italy) and *Trichoderma gamsii* ICC 012 + *Trichoderma asperellum* ICC 080
124 (Remedier, ISAGRO, Italy), which are commercially available. Experimental BCA's have been
125 tested as well: *Trichoderma* sp. TW2 (AgriNewTech, Italy) (Cucu et al., 2018; 2020) and a mixture
126 of *Trichoderma* sp. FC7 and FC8 isolated from suppressive soilless (Agroinnova, Italy) (Garibaldi
127 et al., 2003). In addition, two composts, produced from green waste from a dynamic industrial
128 treatment system, were used: a green compost (Ant's Compost V - CV, AgriNewTech, Italy) and
129 the same compost inoculated with *Trichoderma* sp. TW2 (Ant's Compost M - CM, AgriNewTech,
130 Italy) according to Pugliese et al., (2011). An untreated control was used to monitor *P. capsici*

131 development, while metalaxyl-M (Ridomil gold, 480 g L, Syngenta Crop Protection, Italy) as
132 reference chemical control at 0.48 g a.i. L, label dose concentration, was applied by soil drenching
133 before planting. Summarized details of these treatments can be found in Table 1.
134 Zucchini seeds (cv. Ortano, Syngenta) were sown in 40-plug trays (3.4 cm diameter pots, 4 L of
135 soil, 53 × 42 cm surface) containing a peat mixture substrate.
136 Three soil applications with BCAs were carried out on the plug trays between sowing and
137 transplanting (at the first true leaf stage) in a commercial nursery under glasshouse conditions at
138 temperatures of 20-24°C. The BCAs were applied by spraying them onto the soil surface in a high
139 volume of water (500 ml $tray^{-1}$), using a 1 L capacity hand sprayer. The composts were mixed at
140 20% v/v at the tray level and immediately before sowing (T0).
141 The zucchini plants (15-day old) were transplanted at a density of 3 plants per square meter, drip
142 irrigated and grown according to the cultural practices adopted by commercial growers in the
143 region. The plots were arranged in a completely randomized block design, with five replicates per
144 treatment corresponding to 15 plants per replicate in trials carried out in 2016 and 20 plants per
145 replicate in trials carried out in 2017. The final disease rating was established 62 to 82 days after
146 transplanting on each plot by visually estimate the severity of Phytophthora crown rot symptoms of
147 all the plants present in each plot (Table 2). According to Padley et al. (2008), the disease severity
148 (DS) scale was as follows: 0 = healthy plant, 25% = initial leaf chlorosis, 50% = severe leaf
149 chlorosis and initial symptoms of wilting during the hottest hours of the day, 75% = severe wilting
150 and severe symptoms of leaf chlorosis, 100% = plant totally wilted, leaves completely necrotic.
151 Symptoms were visually estimated at the final evaluation for each plant using a scale rate as follow
152 from 0 to 5.: 0=no symptoms, healthy plants; 1=1 to 30% leaves slight wilted (midpoint 15%); 2=31
153 to 50% foliar wilting with crown lesion (midpoint 40%); 3=51 to 70% of plant is partially collapsed
154 (midpoint 60%); 4=71 to 90% of plant is collapsed (midpoint 80%): 5=over 90% dead plant
155 (midpoint 95%). Disease severity was expressed by using the formula [∑(n° plants × x 0-5) / (total
156 no of plants recorded)] with x 0-5 corresponding to the value reported.

157 2.2. Rhizosphere soil sampling

158 The influence of soil-treatments with BCA's and compost on indigenous microbial communities
159 was studied at the rhizosphere level by collecting samples from both experimental sites at the end of
160 the trials (November 2016 and 2017), as described elsewhere (Cucu et al., 2018). Briefly, one
161 composite rhizosphere sample per plot consisted of the roots of five randomly selected zucchini
162 plants. The roots were shaken vigorously to remove the soil particles not tightly adhering to them.
163 The rhizosphere soil was gently scraped off with sterile forceps and transferred into sterile sampling
164 bags. Each treatment consisted of three biological replicates, which were kept on ice. The samples
165 were then placed in plastic bags, kept on ice and transported to the laboratory.

166

167 2.3. Microbial community analysis

168 2.3.1. Rhizosphere soil DNA extraction

169 A NucleoSpin® Soil kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) was used,
170 according to the manufacturer's instructions, for the rhizosphere soil genomic DNA extraction.
171 Extraction was conducted on 0.6 g sample material. DNA quantity and purity were measured using
172 a NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE, USA) and standardized at 10 ng
173 $\mu l^{-1}$.

174

175 2.3.2 Microbial structure

176 Amplicon target sequencing

177 Total genomic DNA extracted from the rhizosphere samples was used as template to amplify the
178 V3-V4 region of 16S rRNA (primers 16S-F (5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG
179 AGA CAG CCT ACG GGN GGC WGC AG-3') and 16S-R (5'-GTC TCG TGG GCT CGG AGA
180 TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C-3')) (Klindworth et al.,
181 2013) as well as the D1 domain of the 26S rRNA gene (primers LS2-MF 5′-
182 GAGTCGAGTTGTTTGGGAAT-3′ NL4R 5′-GGTCCGTGTTTCAAGACGG-3′) (Mota-Gutierretz

183 et al., 2018). The PCR mix was done in a total volume of 25 μl as follows: 12.5 μl of the 2 × Kapa
184 HiFi HotStart ReadyMix Taq (Roche, Milan, Italy), 5 μl of each primer (1 μM) and 2.5 μl DNA as a
185 template.
186 The PCR mix was subjected to the following amplification cycling : 95°C for 3 minutes; 30 cycles
187 of: 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds and then 72°C for 10 minutes.
188 Amplicons were purified according to the Metagenomic sequencing library preparation of Illumina.
189 Nextera XT Index Kit (Illumina Inc. San Diego. CA) was used based on manufacturer's
190 instructions, to tag each sample. After the 2nd clean up stage, the amplicons were quantified using a
191 QUBIT dsDNA Assay kit and equimolar amounts of amplicons from different samples, belonging
192 to the same target gene, were pooled. The pooled libraries (16S and 26S) were run on an Experion
193 workstation (Biorad, Milan, Italy) for a quality analysis prior to sequencing. The denaturated pool
194 library (with 0.2 N NaOH) was combined with 20% (v/v) of denatured 12 pM PhiX, prepared
195 according to the Illumina guidelines. The sequencing was performed with a MiSeq Illumina
196 instrument (Illumina), with V3 chemistry, and 250 bp paired-end reads were generated, according to
197 the manufacturer's instructions.
198
199 Bioinformatics analysis
200 After sequencing, FLASH software (Magoc et al., 2011) was used to join the paired end reads. Any
201 joined reads shorter than 300 bp were discarded using PRINSEQ. The data were analyzed through
202 QIIME 1.9.0 software (Caporaso et al., 2010). Centroid sequences were used from each Operational
203 Taxonomic Unit (OTU) cluster for the 16S data to assign a taxonomy against the Greengenes 16S
204 rRNA gene database, version 2013, while the in-house database from Mota-Gutierrez et al. (2018)
205 was used for the 26S data.
206 For avoiding biases, due to different sequencing scales, each dataset was reduced at the lowest
207 number of reads: the 26S data were rarefied at 11808 sequences, while the 6285 sequence was
208 chosen for the 16S data. Contamination of chloroplasts was removed from the 16S OTU tables.

209 The 26S OTUs tables generated through QIIME showed the highest taxonomy resolution for the
210 species or genus. The order or class level was instead used for the 16S data. The taxonomy was
211 double-checked using the BlastN search tool (http://www.ncbi.nlm.nih.gov/blast/) to confirm the
212 taxonomy assignment.
213 The data on microbial communities structure were analyzed using the R software (www.r-
214 project.org). The number of different taxa was estimated by sample coverage and microbial α-
215 diversity assessed by Chao1 index. The taxa richness and evenness was calculated by Shannon
216 diversity index, using the *diversity* function of the *vegan* package of R (Dixon, 2003). Beta diversity
217 of 16S data used the phylogenetic distances to compare samples associated with multivariate
218 statistical analysis (Principal coordinate analysis) based on Weighted and Unweighted UniFrac
219 distance matrixes. Anosim and Adonis statistical test, through the *vegan* function in the R
220 environment were used for the 16S data to find any differences between samples, using the
221 phylogenetic distances matrix. A non-parametric Pairwise Wilcoxon test was used, when
222 appropriate, to determine any significant differences in the alpha diversity index or in OTU
223 abundance. Principal component analysis (PCA), based on OTU abundance, was performed and the
224 results were plotted using the *dudi.pca* function of the *made4* package of R. Hierarchical clustering
225 heatplots were obtained, through the *made4* package, using the weighted pair group method
226 together with the arithmetic mean (WPGMA) method. Spearman's pairwise correlations were
227 assessed between the taxa and chemical parameters using the R package *psych*. The P values were
228 adjusted for multiple testing using the Benjamini-Hochberg procedure, which assesses the false
229 discovery rate (FDR). Box plots were used to represent the interquartile range between the first and
230 the third quartiles, with the error bars showing the lowest and the highest values.
231
232 2.3.3 Microbial abundance (quantitative PCR - qPCR)
233 qPCR assays were used to determine the abundance of *P. capisci* (Lan et al., 2013), *Bacillus* spp.
234 (Gao et al., 2011) and *Trichoderma* spp. (Hagn et al., 2007) with the StepOnePlus™ Real-Time

235 PCR System (Applied Biosystems, Foster City, CA, USA). Fungal chitinase *chiA* gene, and the
236 nitrifiers (i.e., bacterial ammonia monooxygenase *amoA* gene - AOB and archaeal *amoA* gene –
237 AOA) have been also evaluated. Table S1 contains a description of the primer sets and
238 amplification details.
239 The standards for the qPCR assays were generated as described by Cucu et al. (2017) from
240 amplicons of each target gene, purified (Invisorb Fragment CleanUp, Stratec Molecular GmbH,
241 Berlin, Germany) and ligated in Strata-Clone PCR cloning vector pSC-A (Strataclone PCR Cloning
242 Kit, Agilent Technologies Inc.); the products of the ligation were then transformed into StrataClone
243 SoloPack competent cells (Agilent Technologies Inc.). The specificity of the amplicons used as
244 qPCR standards was controlled and the Plasmid DNA was isolated (GenElute™ Plasmid Miniprep
245 Kit, Sigma-Aldrich, St. Louis, MO, USA) from standard clones and quantified as described above.
246 *P. capsici*, BCA's, and the bacterial and archaeal *amoA* abundances were determined in a final
247 volume of the qPCR reaction mixtures of 25 µl, while the *chiaA* gene abundance in 20 µl. 10 ng
248 DNA template were used for all quantification reactions. As described earlier by Cucu et al.
249 (2018), the qPCR cocktail had a 1x Power SYBR green master mix (Applied Biosystems), 0.12 µM
250 of each primer (Table S1) for *P. capsici* 0.32 µM for the BCA's, AOB and AOA and 0.4 µM for the
251 *chiA* gene.
252 The quantifications were done with three replicates across plates, while standards were run in
253 duplicate and in 10-fold serial dilutions. Prior quantifications, the best DNA dilution was tested to
254 reduce any inhibitory reactions. Amplification efficiency ranged from 96%, (BCA's and *chiA* gene)
255 to 95.3% and 99.1% for AOB and AOA, respectively tor the nitrifiers. The $R^2$ was always $\geq 0.98$.
256 Melting curves of the amplicons were generated to guarantee the specificity of the reaction and then
257 the amplification products were checked on 1% agarose gel. Always the size of the amplicons was
258 as previously reported by the given protocols (see Table S1). StepOne™ software, version 2.2
259 (Applied Biosystems) was used to calculate the gene copy number and the data were normalized
260 and presented in tables as log copy / g $dw^{-1}$.

261 The data on gene abundance and on the chemical properties of the soil were subjected to a linear
262 mixed model, with time considered as the random variable, using the Statistical Analysis Software
263 program (SAS V 9.2, SAS Institute Inc., North Carolina, USA). The impact of different treatments
264 on the abundance of the considered genes, on the rhizosphere chemical properties and on disease
265 severity were checked out. The Levene test was used to verify the homogeneity of variance, and
266 Shapiro-Wilk test was chosen to check the normality on the residuals; the DS data were arcsin
267 transformed when not normally distributed, while the data from microorganism abundance were
268 log-transformed, and normality checked again. Univariate ANOVA (SPSS 24.09) was used for the
269 data set analyses, and the means were separated by Tukey's multiple comparison test ($p = 0.05$).
270 The statistical analysis included the treatment, year and treatment × year. A least square means
271 comparison between treatments was conducted using Tukey's range test ($p < 0.05$). Pearson linear
272 correlation analyses were conducted, and the results were visualized to establish linearity in the
273 SAS COR procedure in order to relate the abundance of the target genes (dependent variables) to
274 the chemical properties of the soil (independent variables).
275
276 2.4. Geo - chemical characterization of the rhizosphere samples
277 The fresh rhizosphere samples were homogenized, passed through a 2 mm sieve, stored at 4°C and
278 analyzed within 24 h. All the samples were split into two. One part was analysed at the Regional
279 Chemistry Laboratory (Turin, Italy) for humidity, pH, total N (TN), inorganic N as nitrate ($NO_3^-$)
280 and ammonium ($NH_4^+$), total phosphorus (Pt) and available phosphorus (Pav), while the second part
281 was used for molecular investigations.
282 The method for measuring pH was in water suspensions with solid: liquid ratio of 1:2.5. A Leco Tru
283 Spect CN automatic analyzer was used for the total nitrogen (TN) quantification. Inorganic
284 nitrogen, ammonium ($NH_4^+$) and nitrate ($NO_3^-$) using standard colorimetric techniques by means of
285 a continuous flow auto-analyzer (Alliance Evolution II). The total phosphorus (Pt) was determined
286 by means of 'ICP Varian mod. Liberty LR', after microwave based digestion with hydrogen

287 peroxide, hydrochloric acid and nitric acid, filtration and dilution. The available P (Pav) was
288 determined by means of the molybdenum blue method (Olsen et al., 1954), modified for continuous
289 flow colorimetric analysis (Alliance Evolution II).

290

291 3. Results

292 3.1 Disease severity (DS)

293 The obtained results showed that the application of BCAs and certain organic matter can effectively
294 suppress soil pathogens and reduce the disease. The disease severity (DS) in the non-treated control
295 plots was higher in Carmagnola, where the soil was artificially infested with the pathogen, than in
296 Moretta for both years (Table 2). During 2016, all the treatments based BCAs and compost
297 significantly reduced the disease from 48 to 62% in the Carmagnola site and from 52 to 80% in the
298 Moretta site, compared to the untreated plots (Table 2). In 2017, the artificial infestation of soil with
299 the pathogen at the Carmagnola site produced a greater disease severity (DS 78% in the untreated
300 control) resulting in a significant Phytopthora crown rot reduction between 32 to 49% provided by
301 all the treatments, apart from the *Trichoderma* spp. T and TW2 treatments. While, in the Moretta
302 site, under a low disease pressure (DS 28% in the untreated control) all the tested treatments
303 provided a significant disease reduction from 50 to 82%. The reference chemical treatment based
304 metalaxyl-M was the most effective, reducing the DS by 56-78% in Carmagnola and by 94-99% in
305 Moretta in both years. The efficacy of the two composts (CV and CM) was statistically similar to
306 that of the chemical fungicide in both locations, reducing the DS by 56-59% and 45-49% in
307 Carmagnola and by 69-80% and 73-82% in Moretta, respectively. (Table 2).

308

309 3.2. Microbial diversity

310 The microbiota and mycobiota diversity in the two sites for the two different years was
311 characterized by 16S and 26S rRNA gene sequencing, obtained from gDNA extracted directly from
312 the rhizosphere soil samples. The DNA concentration which ranged between 82-100 ng/g $dw^{-1}$ for

313 Moretta and 55 - 83 ng/ g $dw^{-1}$ for Carmagnola, in both years, had the ratio of the absorbance at
314 260 nm and 280 nm of ~1.8, assessing a "pure" DNA. Overall, 1,907,342 and 3,849,188 high
315 quality reads were analyzed for 16S and 26S, respectively. The alpha-diversity indexes (Shannon,
316 Chao1 diversity index) and the number of species observed on the basis of OTUs of 97% identity,
317 are shown in Figure 1A (16S) and Figure 1B (26S). Anosim and Adonis statistical test performed
318 on 16S as well as 26S showed no difference for the different BCA's treatments and composts in the
319 two soils in the two years ($P>0.05$).
320 As far as the 16S data are concerned, the Carmagnola 2017 soil displayed a significantly higher
321 microbial diversity and richness ($P < 0.001$) than Carmagnola 2016 and than the other site.
322 Moreover, the Moretta 2017 samples showed higher richness than the Moretta 2016 ones ($P < 0.05$)
323 - see Figure 1A.
324 As for the mycobiota (Figure 1B), it was possible to observe that the Chao1 index (an estimator of
325 species richness) and the number of observed OTUs were higher in the Moretta samples than in the
326 Carmagnola ones ($P < 0.001$). However, no differences were observed between Carmagnola 2016
327 and Carmagnola 2017 or between Moretta 2016 and Moretta 2017. It should be pointed out that the
328 different treatments did not affect the microbial diversity.
329 Using a Principal Coordinate analysis (PCoA, Fig. 2) of the weighted UniFrac distance matrix, we
330 observed a clear separation of the microbiota between Carmagnola 2016 vs. Carmagnola 2017 and
331 Moretta 2016 vs. Moretta 2017 (ANOSIM statistical test $P < 0.001$) for the beta diversity
332 calculation of the 16S data. No differences were observed between Carmagnola 2016 and Moretta
333 2016. Samples from these two sites from the same year clustered together and were well separated
334 from the others (Fig. 2).
335 A similar trend was observed for the mycobiota populations. The principal component analysis
336 (PCA), based on the 26S OTU table, clearly showed a separation of Carmagnola 2017 from the
337 other site, while a distribution of the other samples was less marked (ANOSIM statistical test P <

338 0.001) (Figure S1). No differences were observed when the different soil treatments were taken into
339 account.
340
341 *3.2.a Microbiota composition*
342 The relative abundances of the bacterial taxa were analyzed at a class level to show the
343 development of the microbiota over time, in both years, and across sites. A total of 34 classes, with
344 a relative abundance of 0.2% in at least 10 samples, were observed (Table 3). When the
345 Carmagnola site was considered in 2016 and 2017 (Table 3), the microbiota were dominated by the
346 presence of *Actinobacteria* (median value of 6 and 13% of the relative abundance, respectively),
347 *Alphaproteobacteria* (11 and 13%), *Bacilli* (5 and 7%), *Betaproteobacteria* (6 and 4%), *Chloroflexi*
348 (3 and 5%), *Deltaproteobacteria* (5 and 4%), *Phycisphaerae* (5 and 3%), *Planctomycetia* (9 and
349 6%) and *Thermoleophilia* (3 and 6%) - see Table 3.
350 The same trend was observed for the Moretta samples (Table 4), where *Actinobacteria* showed a
351 relative abundance, with a median value of 6 and 13% in 2016 and 2017, respectively;
352 *Alphaproteobacteria* (12 and 12%), *Bacilli* (3 and 7%), *Betaproteobacteria* (5 and 4%), *Chloroflexi*
353 (3 and 5%), *Deltaproteobacteria* (5 and 4%), *Gammaproteobacteria* (7 and 4%), *Phycisphaerae* (6
354 and 4%), *Planctomycetia* (5 and 7%) and *Thermoleophilia* (2 and 6%).
355 We observed a specific microbiota signature among the variables from a the pairwise comparison of
356 the sites and the years. In particular, *Actinobacteria*, *Bacilli* and *Chloroflexi* were found to be
357 associated (FDR $< 0.05$) with Carmagnola 2016 and Moretta 2016; *Betaproteobacteria*,
358 *Deltaproteobacteria* and *Phycisphaerae* with Carmagnola 2017 and Moretta 2017;
359 *Alphaproteobacteria* was observed to be associated with Carmagnola 2016 and
360 *Gammaproteobacteria* with Moretta 2017 (Figure S2).
361
362 *3.2.b Mycobiota composition*

363 A total of 58 taxa, with a relative abundance of 0.2% in at least 10 samples, were observed (Tables
364 S2 and S3). When only the main fungi that occurred with a higher percentage than 5% were taken
365 into account, the presence of *Alternaria tenuissima* (median values of 1 and 9% of the relative
366 abundance in 2016 and 2017, respectively), *Cladosporium cladosporioides* (median value sof 4 and
367 9%), *Fusarium oxysporum* (6 and 3%), Leotiomycetes (19 and 2%) and *Pseudeurotium zonatum*
368 (28 and 1%) was observed in the Carmagnola site in 2016 and 2017 (Table S2)
369 The Moretta samples (Table S3) were dominated by the presence of *Aspergillus oryzae* (median
370 values of 5 and 1% of the relative abundance in 2016 and 2017, respectively), Leotiomycetes (13
371 and 23%) and *Pseudeurotium zonatum* (34 and 35%).
372 A specific mycobiota signature between the sites and the years was observed from the pairwise
373 comparison (Figure S3, FDR < 0.05). In particular, it was observed that *Alternaria tenuissima* and
374 *Cladosporium cladosporioides* were associated with the Carmagnola site in 2017, *Aspergillus*
375 *oryzae* and *Fusarium oxysporum* with the Carmagnola site in 2016, Leotiomycetes were associated
376 with both sites in 2016 and 2017 and *Pseudeurotium zonatum* was associated with the Carmagnola
377 site in 2016 and Moretta 2016 (Figure S3, FDR < 0.05).
378
379 3.3. Microbial abundance
380 *3.3a Abundance of soil-borne* P. capsici *by means of qPCR*
381 All the treatments resulted in a significant pathogen reduction ($p < 0.05$), compared to the untreated
382 control (C), in both the Moretta and Carmagnola sites, in both years. The quantity of the pathogen
383 in the Moretta rhizosphere samples was lower after the RM treatment in both years, while it was
384 lower in the Carmagnola site after the RM treatment in 2016 and 2017 respectively (Table 5).
385
386 *3.3b Abundance of antagonists N- cycling genes by means of qPCR*
387 All the treatments resulted in a significant boost of *Bacillus* spp. and *Trichoderma* spp., compared
388 to the untreated control - C. The *Bacillus* spp. abundance was higher after the *Bacillus* - SM

389 treatment and lower after the *Trichoderma* sp. - T treatment and *Trichoderma* sp. - TW2 in the
390 Moretta samples in 2016 and 2017 respectively, while the *Bacillus* spp. abundance was higher after
391 the *Bacillus* - SM treatment and lower after the *Trichoderma* sp. - TW2 treatment for the
392 Carmagnola counterparts after both years (Table 6).
393 *Trichoderma* spp. abundance significantly enhanced after all the *Trichoderma* sp. treatments,
394 compared with the untreated control - C for both sites and both years (Table 6).
395 The same trend was observed for *chiA* gene abundance, which significantly enhanced after all the
396 *Trichoderma* spp. treatments, compared with the untreated control - C and the *Bacillus* treatment -
397 SM in Moretta samples after both years. However, the enriched compost – CM had the highest
398 abundance of the *chiA* gene. Carmagnola samples were characterised by the same situation, but with
399 the highest abundance of *chiA* gene after *Trichoderma* sp. - TW2 in 2016 and enriched compost –
400 CM in 2017 (Table 7).
401 The ammonia-oxidizing bacterial (AOB) and archaeal (AOA) gene abundance was significantly
402 influenced by treatments. Both sites were characterized by the increase of AOB and AOA
403 abundance in all treatments comparing with the untreated control. In general, the abundance of
404 AOB was higher in rhizosphere samples from Carmagnola site, while the abundance of AOA was
405 higher in rhizosphere samples from Moretta site (Table S4 and Table S5).
406

407 3.4. Chemical properties
408 The pH values of rhizosphere samples generally were lower for Moretta site than for Carmagnola
409 ones and ranged from between 5.36 to and 6.23 and 6.20 to 7.78 respectively. The untreated control
410 was characterized by higher $NH_4^+$, $NO_3^-$ concentrations than all the treatments. On the other hand,
411 the Pt and Pav contents were lower (Table S6).
412

413 3.5. Correlations
414 3.5 a. Spearman's pairwise correlations assessed between the taxa and chemical parameters

415 By plotting the correlation between chemical determination, microbiota and mycobiota in
416 Carmagnola samples (Figure S4A; FDR < 0.05) the results showed more relationships between the
417 taxa and TN, $NO_3^-$ and $NH_4^+$ with respect to Moretta samples (Figure S4B; FDR < 0.05). By
418 plotting the correlation between chemical determination, microbiota and mycobiota in Moretta
419 samples for both years (Figure S4B; FDR < 0.05) the results showed more relationships between
420 the taxa and pH as well as between the taxa and the pathogen abundance.
421

422 b. Pearson correlations between *Bacillus* and *Trichoderma* abundances and chemical
423 parameters
424 In general, the correlations with pH were positive, with r coefficients ranging from 0.55 to 0.85 ($p <$
425 $0.001$) for the rhizosphere samples from Moretta e Carmagnola sites. The correlations were
426 negative between *Bacillus* and Pt and Pav and positive for *Trichoderma* (Table S7).
427

428 4. Discussion
429 Four biocontrol agents and two compost were chosen to control soil borne pathogens as *P. capsici*.
430 It is well known that their effect is relative, depending on the plant host, involved pathogen species
431 and the characteristics of the applied treatments (Bonanomi et al., 2006). Even more, the
432 interactions among beneficial microorganisms newly introduced into the soil system, as single
433 strains or in microbial consortium, with indigenous rhizosphere microbial populations and the plant
434 usually vary with the environmental conditions (Mendes et al., 2013).
435 The results of the field experiments showed that the all treatments reduced the disease severity and
436 were particularly effective against the pathogen, in comparison to the untreated control, (Heydari
437 and Pessarakli, 2010; Noble and Coventry 2005; Pugliese et al., 2015). The findings were in line
438 with the fact that pathogens populations in the soils could be reduced by biocontrol agents (Huang
439 et al., 2011; Wei et al., 2011) and suppressive compost (Cucu et al., 2018). Similarly, previous
440 studies showed several bacilli strains being effective against Phytophthora blight caused by *P.*

441 *capsici* on squash (Zhang et al., 2010; Gilardi et al., 2015) and on pepper (Sang et al, 2013). Even
442 more, the mixture of organic substrates and BCA's may induce the suppression of plant pathogens
443 through different mechanisms. Blaya et al. (2013), Cao et al. (2011) and Hoitink et al. (2006)
444 hypothesized the presence of direct interactions as competition for nutrients and space, the
445 production of antibiotics, mycoparasitism and indirect interactions, such as systemic and acquired
446 resistance (ISR and SAR). Nevertheless, the disease severity was greater at higher pH conditions
447 which was in agreement with previous studies involving *P. capsici* as pathogen (Muchovej et al.,
448 1980).
449 Through the use of the targeting approach, a reduction in *P. capsici* abundance was observed as a
450 result of all the applied treatments, thereby assuming that a direct pathogen-BCA's interaction
451 occurred. This effect could be due to antibiosis or/and mycoparasitism process induced by
452 *Trichoderma* spp. hydrolytic enzyme secretion, which may have caused the hydrolysis of the
453 pathogen cell wall, or antibiotic compound production (Woo et al., 2006, Lorito and Woo, 2015a).
454 A higher abundance of *Trichoderma* spp. and of the *chiA* gene in all the treatments was observed
455 highlighting the positive effect of *Trichoderma* spp. when used as a biocontrol agent or in
456 combination with organic material as pointed out by Lorito et al. (2010), Hermosa et al. (2012), and
457 Cucu et al. (2018). This result in corroboration with good relationships with rhizosphere chemical
458 parameters evidenced the fact that *Trichodema* spp. readily transfers and absorbs soil nutrients (e.g.,
459 phosphate - Tandon et al., 2018) and is more efficient and competitive that many other fungi. In
460 consequence, it can therefore be used effectively to improve the soil structure and promote the
461 establishment and maintenance of other beneficial microorganisms (Benitez et al., 2004). The
462 targeting analyses showed also a good feedback of *Bacillus* spp. after all the treatments. The same
463 response was observed by Cucu et al. (2018) in a *Fusarium oxysporum* - lettuce pathosystem,
464 evidencing a good feedback of indigenous rhizosphere *Bacillus* populations. In general, *Bacillus*
465 spp. was found to be extremely effective in plant disease suppression as it forms a stable and
466 extensive biofilm (Bais et al. 2004) composed of secreted antifungal compounds, such as surfactin,

467 bacillomycin and microlactin, which protect plants from attack by soil-borne pathogens (Bais et al.
468 2004; Yuan et al. 2012). Even more, Yang et al. (2012) and Sid et al. (2003) reported that most
469 species of Bacillus were able to inhibit the mycelia growth of *P. capsici* effectively *"in vitro"*.
470 Through the use of the un-targeting approach (amplicon based sequencing), it was shown also that
471 the different BCA's and compost treatments did not affect the composition of the resident
472 microbiota and mycobiota. This implied that the used treatments did not have any effect on the total
473 microbial communities reflecting the great ecological stability of the rhizosphere (Thébault and
474 Fontaine, 2010). In turn suggested a slow but good adaptation of the BCA's to the environment.
475 Anyway, the observed differences among sampling dates may suggest that the composition of the
476 soil microbial community might have been altered by differences in temperature and precipitation,
477 as response of the microbial groups to environmental variables.
478 *Actinobacteria*, *Bacilli* and *Chloroflexi* were the most abundant bacterial phyla in both sites in 2016,
479 while *Betaproteobacteria*, *Deltaproteobacteria* and *Phycisphaerae* were observed in both of the
480 studied sites in 2017; *Alphaproteobacteria* was associated with the Carmagnola site in 2016, while
481 *Gammaproteobacteria* was associated with the Moretta site in 2017. Soil microbial communities
482 structure was shaped by a multitude of factors, including environmental parameters as suggested by
483 the correlation analyses of soil chemical properties namely TN, $NO_3^-$-N, $NH_4^+$-N available P and
484 the relative abundances of different microbial populations across the considered sites, especially for
485 the Carmagnola rhizosphere soil. This observed differences could be attributed to the difference in
486 the soil pH, as it is generally considered as the best predictor of the microbial community
487 composition and diversity (Fierer and Jackson, 2006). The similarity of the chemical and physical
488 characteristics of the individual soil sites permitted to consider independently the pH effects. This
489 was confirmed by the abundance data of populations of ammonia-oxidizing bacteria and archaea.
490 The results showed an elevated amount of ammonia-oxidizing bacteria, mainly *Betaproteobacteria*,
491 with respect to the untreated control especially at higher pH of Carmagnola site, in line with what
492 previously reported (Nicol et. al., 2008). On the opposite a higher abundance of archaeal ammonia

493 oxidizers was observed in samples from Moretta site this suggesting a potential niche differentiation
494 between the ammonia oxidizers as indicated also by the relationships with chemical properties
495 (Figure S4A and B). Although the increase of ammonia-oxidizing bacteria and archaea abundance
496 with respect to the untreated control may suggest a possible contribution of these microorganisms to
497 the disease reduction, further research in regard must be taken in consideration.
498 The Moretta site showed a predominance of relationships between the pH and microbial taxa
499 (Figure S4B). Prior to the use of NGS, Fierer and Jackson (2006), using Terminal Restriction
500 Length Polymorphism, identified a maximum of microbial diversity for a neutral pH. Rousk et al.
501 (2010) extended this finding using experimental plots. Sequences that were classified as belonging
502 to *Gammaproteobacteria* were shown to correlate negatively with pH, a result that was in
503 contradiction with what Postma et al. (2011) reported. On the other hand, the relative abundance of
504 *Actinobacteria* was found to be positively correlated with pH. Lauber et al. (2009) also reported
505 clear shifts in the bacterial-phylum level, with the relative abundance of *Actinobacteria* increasing
506 as the pH value increased.
507 The soil artificially infested with *P. capisici* showed a somewhat increased abundance of
508 *Actinobacteria*. Ros et al. (2016) observed the same phenomenon after seedling infection by *P.*
509 *nicotianae*. This result, together with good correlations with the soil properties (Figure S4A),
510 indicated that the highlighted microbial communities could play a strategic role in the organic
511 materials decomposition and in the nutrient cycle, as evidenced by Steger et al. (2003). Although
512 these microorganisms have a great ability to produce antibiotic-like compounds (McKellar and
513 Nelson, 2003), the results indicated that they did not have a positive influence on plant disease
514 suppression in the case of an artificial infestation since no relationship with *P. capsici* was found.
515 The opposite was observed for the natural infestation, result evidenced by correlations between
516 *Actinobacteria* and pathogen. Thampi and Bhai, (2017) reported some *Actinobacteria* strains as
517 potential antagonists against *P. capsici*, after their isolation and characterization from rhizosphere
518 samples of black pepper. However, these results might be contradictory as Sutherland and

519 Papavizas (1991) reported, in a very early “in vitro” study, that *Actinobacteria*, which infested
520 oospores of *P. capsici*, were not effective under greenhouse conditions and didn’t control the crown
521 rot of pepper. Cuesta et al. (2012) also reported that the presence of *Actinobacteria* is typical of
522 suppressive substrates. Bonanomi et al., (2010) concluded that disease suppression was only
523 correlated with *Actinobacteria* in a small number of experiments. However, the here presented
524 results suggested that enlarged populations of Actinomycetes might lead to a favourable
525 environment for biocontrol strains against *P. capsici* on zucchini.
526 *Bacillus* was found as some of the most abundant genera in all the treatments, the sequencing
527 results being supported by the quantitative analyses. The observed relatively high abundance of
528 *Bacilli* phyla was in accordance with several other studies which reported the potential antagonism
529 of *Bacillus* species against *P. capsici*. For example, Lee et al., (2008), on the basis of 16S rDNA
530 sequencing, showed that the most effective isolates with antagonistic activity against *P. capsici*
531 were *Bacillus subtilis.* In addition, differences were observed between the studied sites regarding
532 the relationships between the soil parameters and the pathogen, results which indicated that the
533 presence of *Bacillus* was induced by a natural infestation with the pathogen at a neutral pH. This
534 may explain the effectiveness of *Bacillus* spp. in the presence of a native pathogen. The *Bacillus*
535 treatment was also effective in the case of artificially infested soil, as evidenced by Li et al. (2019).
536 The good correlations between the relative abundance of *Bacillus* and the soil properties in the
537 artificially infested soil indicated the important effects of *Bacillus* spp. on nutrient availability and
538 plant growth, as already described by Charest at al. (2005) and Garcia et al. (2011).
539 The interaction between *P. capsici* – BCA’s strains at the rhizosphere level showed the presence of
540 *Alphaproteobacteria* enrichment and, in particular, the more relative abundance of *Bradyrizobium*,
541 *Mesorhizobium*, *Hypomicrobium*, thus indicating that they may be involved in the disease
542 suppression of *P. capsici*. As pointed out by Compant et al., (2010), rhizobia may play a very
543 important role in biocontrol, due to the secretion of antibiotics and hydrogen cyanide (HCN), and
544 also due to the production of siderophores.

545 The fungal community was characterised by the abundant presence of Ascomycota after all the
546 treatments administered including the compost ones, as also shown by Blaya et al., (2016) and
547 Langarica et al., (2014). The variation of the mycobiota at a phylum level was probably principally
548 due to the type of substrate rather than to the pathogen infection. It should be considered that
549 different genera, such as *Trichoderma* spp. and *Fusarium* spp., were abundant in all the treatments,
550 independently of the pathogen infection. *Trichoderma*, *Fusarium* and *Cladosporium* species have
551 been considered as biological control agents (Kaur et al., 2010, Lopez-Mondeja et al., 2010) and
552 can be speculated to have contributed to the disease suppression to a great extent. The fungal
553 species belonging to Leotiomycetes (Wang et al., 2006) have the potential to improve nutrient
554 acquisition and combat pathogenic taxa, maintaining cooperative metabolic associations with other
555 species. Species of *Aspergillus* and *Pseudoritium*, the potential phosphate-solubilising fungal
556 genera, were also detected in this study. Nevertheless, further research must be considered to better
557 understand the functional composition of fungal community as a wide range of fungi may be
558 involved effectively in the pathogen control. In addition, the temperature, soil moisture and soil
559 type must be taken into account as this are among the most import factors that affect the
560 composition and structure of a microbial community (Spadaro and Gullino, 2005; Cregger et al.,
561 2015).
562 However, to better understand the effects of the different used treatments, it is necessary to focus on
563 the functional mechanism induced by the interaction of the pathogen with the BCA's newly
564 introduced into the system and dictated by the chemistry soil parameters, particularly the pH.
565 Hence, further studies are necessary considering several soil types characterized by contrasting
566 physico-chemical parameters.
567

568 **Conclusions and outlook**

569 The use of *Bacillus subtilis* and *Trichoderma* spp., and two different composts has been effective in
570 reducing *P. capsici* abundance and the disease severity at both experimental sites in naturally and

571 artificial infested soils. In addition, the results have shown that, in general, the *Bacillus subtilis* and
572 *Trichoderma* spp. based treatments resulted in a good feedback of the resident *Trichoderma* spp.
573 and *Bacillus* spp. communities from rhizosphere. After applied BCA's treatments, the antagonists
574 established itself in soil together with the same resident populations used the nutrients from the
575 organic matter which boosted their population and made them more competitive in the soil. The
576 disease suppression was probably BCA's functionally induced and not controlled by changes in the
577 microbial structure or diversity. The differences in the rhizosphere bacterial community in both sites
578 were not attributed to the treatments, but rather to the soil pH and to the origin of the pathogen
579 infection. On the other hand, the fungal community was found to depend on the type of rhizosphere
580 as substrate, but was not affected by the modality of plant infection (e.g., naturally or artificially) .
581 However, the complex phenomenon of disease suppressiveness of the studied soils cannot simply
582 be ascribed to a single bacterial or fungal taxon, or group, but is most likely governed by microbial
583 consortia.
584 These results suggested that the next-generation sequencing approaches is useful to investigate
585 microbial interactions, as essential knowledge may be acquired on the impact that beneficial
586 microorganisms have on non-targeted microbial communities in the rhizosphere, while controlling
587 the pathogen. Thus, the modern approach of this study has highlighted and confirmed the potential
588 of *Bacillus subtilis* and *Trichoderma* spp. used alone or in microbial consortia in combination also
589 with organic material, as biocontrol agents for a sustainable management of Phytophthora crown rot
590 of zucchini, since the disease severity was reduced without causing any significant change in the
591 resident microbial communities under the considered field conditions.
592

593 **Acknowledgements**
594 The project leading to this application has received funding from the European Union's Horizon
595 2020 research and innovation programme under grant agreement No 634179 - EMPHASIS. The
596 authors thank Professor Marguerite Jones for the language revision.

597 **Conflict of Interest**

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

601

602

603 **References**


604 Bais, H.P., Fall, R., Vivanco, M., 2004. Biocontrol of *Bacillus subtilis* against infection of
605 Arabidopsis roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin
606 production. ASPB. 134, 307-319.
607 Benítez, T., Rincón, A.M., Limón, M.C., Codón, A.C., 2004. Biocontrol mechanisms of
608 *Trichoderma* strains. Int. Microbiol. 7, 249-260.
609 Blaya, J., López-Mondéjar, R., Lloret, E., Pascual, J.A., Ros, M., 2013. Changes induced by
610 *Trichoderma harzianum* in suppressive compost controlling Fusarium wilt. Pestic. Biochem.
611 Phys. 107, 112-119.
612 Blaya, J., Marhuenda, F., Pascual, J., Ros, M., 2016. Microbiota characterization of compost using
613 omics approaches opens new perspectives for Phytophthora root rot control. PLosONE 11,
614 e0158048.
615 Bonanomi, G., Antignani, V., Capodilupo, M., Scala, F., 2010. Identifying the characteristics of
616 organic soil amendments that suppress soilborne plant diseases. Soil Biol. Biochem. 42,
617 136-144.
618 Bonanomi, G., Giorgi, V., Giovanni, D.S., Neri, B., Scala, F., 2006. Olive mill residues affect
619 saprophytic growth and disease incidence of foliar and soilborne plant fungal pathogens.
620 Agric. Ecosyst. Environ. 115, 194-200.
621 Bonanomi, G., Lorito, M., Vina,le F., Woo, S.L., 2018. Organic amendments, beneficial microbes
622 and soil microbiota: toward a unified frame work for disease suppression. Annu. Rev.
623 Phytopatol. 56, 1-20.
624 Cao, F.Y., Yoshioka, K., Desveaux, D., 2011. The roles of ABA in plant - pathogen interactions. J.
625 Plant Res.124, 489-499.
626 Caporaso, J. G. et al., 2010. QIIME allows analysis of high-throughput community sequencing data.
627 Nat. Meth. 7, 335-336.
628 Chae, D.H., Jin, R. D., Hwangbo, H., Kim, Y.W., Kim, Y.C., Park, R. D., Krishnan, H.B., Kim,
629 K.Y., 2006. Control of late blight (*Phytophthora capsici*) in pepper plant with a compost
630 containing multude of chitinase-producing bacteria. BioContr. 51, 339-351.
631 Charest, M.H., Beauchamp, C.L., Antoun, H., 2005. Effects of the humic substances of deinking
632 paper sludge on the antagonism between two compost bacteria and *Pythium ultimum*. FEMS
633 Microbiol. Ecol. 5, 219-227.
634 Chen, J.T., Su, H.J., Huang, J.W., 2012. Isolation and identification of secondary metabolites of
635 *Clitocybe nuda* responsible for inhibition of zoospore germination of *Phytophthora capsici*.
636 J. Agr. Food Chem. 60, 7341-7344.
637 Colla, P., Gilardi, G., Gullino, M.L., 2012. A review and critical analysis of the European situation
638 of soilborne disease management in the vegetable sector. Phytoparasitica 40, 515-523.

639 Compant, S., Clément C. and Sessitsch, A.. 2010. Plant growth promoting bacteria in the rhizo and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. Soil Biol. Biochem. 42, 669-678.
642 Cregger, M.A., Sanders, N.J., Dunn, R.R., Classen, A.T., 2015. Microbial communities respond to experimental warming, but site matters. PeerJ 2, e358.
644 Cucu, M.A., Marchan, S., Said-Pullicino, D., Celi, L., Kandeler, E., Rasche, F., 2017. Resource driven community dynamics of $NH_4^+$ assimilating and $N_2O$ reducing archaea in a temperate paddy soil. Pedobiologia, 62, 16-27.
647 Cucu, M.A., Gilardi, G., Pugliese, M., Matic, S., Ulrich, G., Gullino, M.L., Garibaldi, A., 2018. Influence of different biological control agents and compost on total and nitrification driving microbial communities at rhizosphere and soil level in a lettuce - *Fusarium oxysporum* f. sp. *lactucae* pathosystem. J. App.Microb. doi: 10.1111/jam.14153.
651 Cucu M.A., Gilardi G., Pugliese M., Gullino M.L., Garibaldi A., 2020. 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. Biol. Control, 142, 1-10.
655 Cuesta, G., Garcia-de-la-Fuente, R., Abad, M., and Fornes, F., 2012. Isolation and identification of actinomycetes from a compost-amended soil with potential as biocontrol agents. J. Environ. Manage. 95, S280-S284.
658 da Costa Ribeiro, C.S. and P.W. Bosland. 2012. Physiological race characterization of *Phytophthora capsici* isolates from several host plant species in Brazil using New Mexico Recombinant Inbred Lines of *Capsicum annuum* at two inoculum levels. J. Amer. Soc. Hort. Sci. 137, 421-426.
662 Dixon, P., 2003. VEGAN, a package of R functions for community ecology. J. Veg. Sci. 14, 927-930.
664 Ezziyyani, M., Requena, M.E., Egea-Gilabert, C., et al., 2007. Biological control of Phytophthora root rot of peppercili using *Trichoderma harzianum* and *Streptomyces rochei* in combination. J. Phytopathol. 155, 342-349.
667 Fierer, N. & Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. P. Natl. Acad. Sci. USA 103, 626-631.
669 Francis, C.A., Roberts, K.J., Beman, J.M., Santoro, A.E., Oakley, B.B., 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proc. Natl. Acad. Sci. USA 102, 14683-14688.
672 Gao, W., Zhang, W. and Meldrum, D.R., 2011. RT-qPCR based quantitative analysis of gene expression in single bacterial cells. J. Microbiol. Methods, 85, 221-227.
674 Garcia, J.L., Probanza, A., Ramos, B., Manero, F.J.G., 2011. Ecology, genetic diversity and screening strategies of plant growth promoting rhizobacteria. J. Plant Nutr. Soil Sci. 164,1-7.
676 Garibaldi, A., & Gullino, M.L., 2010. Emerging soilborne diseases of horticultural crops and new trends in their management. Acta Horticolturae, 883,37-47.
678 Garibaldi, A., Guglielmone, L., Gullino, M.L., 1989. Rhizosphere competence of antagonistic *Fusaria* isolated from suppressive soils. Symbiosis, 9, 401-404
680 Garibaldi, A., Minuto, A., Grasso, V., Gullino, M.L., 2003. Application of selected antagonistic strains against *Phytophthora cryptogea* on gerbera in closed soilless systems with disinfection by slow sand filtration. Crop Prot. 22,1053-1061.
683 Gilardi, G., Baudino, M., Moizio, M., Pugliese, M., Garibaldi, A., Gullino, M. L., 2013. Integrated management of *Phytopthora capsici* on bell pepper by combining grafting and compost treatment..Crop Prot. 53, 13-19.
686 Gilardi, G., Demarchi, S., Gullino, M. L., Garibaldi, A., 2015. Nursery treatments with non-conventional products against crown and root rot, caused by *Phytophthora capsici*, on zucchini. Phytoparasitica, 43, 501-508.

689 Gupta, V. K., Schmoll, M., Herrera-Estrella, A., Upadhyay, R. S., Druzhinina I., Tuohy, M.G.,
690 2014. Biotechnology and Biology of *Trichoderma*. Oxford: Elsevier Science and
691 Technology; 10.1016/C2012-0-00434-6.
692 Hagn, A., Wallisch, S., Radl, V., Munch, J.C. and Schloter, M., 2007. A new cultivation
693 independent approach to detect and monitor common *Trichoderma* species in soils. J.
694 Microbiol. Methods, 69, 86-92.
695 Harman, G.E., 2000. Myths and dogmas of biocontrol: changes in perceptions derived from
696 research on *Trichoderma harzianum* T-22. Plant Dis. 84 377-393.
697 10.1094/PDIS.2000.84.4.377.
698 Harman, G.E., Howell, C.R., Viterbo, A., and Chet, I., 2004. *Trichoderma* spp. - Opportunistic
699 avirulent plant symbionts. Nat. Rev. Microbiol. 2,43-56.
700 Hausbeck, M.K., Lamour, K., H., 2004. *Phytophthora capsici* on vegetable crops: research progress
701 and management challengers. Plant Dis. 88,1292-1303.
702 Hermosa, R., Viterbo, A., Chet, I., Monte, E., 2012. Plant-beneficial effects of *Trichoderma* and of
703 its genes. Microbiol. 158,17-25. 10.1099/mic.0.052274-0.
704 Heydari A., Pessarakli M., 2010. A review on biological control of fungal plant pathogens using
705 microbial antagonists. J. Biol. Sciences, 10, 273-290.
706 Hoitink, H.A.J., Madden, L.V., Dorrance, A.E., 2006. Systemic resistance induced by *Trichoderma*
707 spp.: Interactions between the host, the pathogen, the biocontrol agent, and soil organic
708 matter quality. Phytopathol. 96,186-189.
709 Howell, C.R., 2003. Mechanisms employed by *Trichoderma* species in the biological control of
710 plant diseases: the history and evolution of current concepts. Plant Dis. 87,4-10.
711 Huang, X., Chen, L., Ran, W., Shen, Q., Yang, X., 2011. *Trichoderma harzianum* strain SQR-T37
712 and its bio-organic fertilizer could control *Rhizoctonia solani* damping-off disease in
713 cucumber seedlings mainly by the mycoparasitism, Appl. Microbiol. Biotechnol. 91, 741-
714 755.
715 ISTAT, 2017. http://dati.istat.it/
716 Jeffrey, C., 2001. Cucurbitaceae. In P. Hanelt, ed., Mansfeld's encyclopedia of agricultural and
717 horticultural crops. Springer, Berlin, pp 1510-1557
718 Jiang, H., Zhang, L., Zhang, J.Z., Ojaghian, M.R., Hyde, K.D., 2016. Antagonistic interaction
719 between *Trichoderma asperellum* and *Phytophthora capsici* in vitro. J Zhejiang Univ Sci B.
720 17, 271-281.
721 Kang, S. & Kim, S., 2004. New antifungal activity of penicillic acid against *Phytophthora* species.
722 Biotech. Letters, 26,695.
723 Kaur, S., Singh, N., Saudhu, P.S., 2010. In vitro evaluation of *Trichoderma viride* and *T. harzianum*
724 against *Macrophomina phaseolina* causing charcoal root rot of sunflower. Plant Dis. Res.
725 25,79-89.
726 Kim, K.D., Nemec, S., Musson, G., 1997. Control of Phytophthora root and crown rot of bell
727 pepper with composts and soil amendments in the greenhouse. Appl. Soil Ecol. 5,109-179.
728 Kim, S.G., Z. Khan, Y.H. Jeon and Y.H. Kim., 2009. Inhibitory effect of *Paenibacillus polymyxa*
729 GBR-462 on *Phytophthora capsici* causing Phytophthora blight in chili pepper. Phytopathol.
730 J. 157, 329-337.
731 Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O., 2013.
732 Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-
733 generation sequencing-based diversity studies. Nucleic acids res., 41(1), e1.
734 doi:10.1093/nar/gks808.
735 Lamour, K.H., Stam, R., Jupe, J., Huitema, E., 2012. The oomycete broad-host range pathogen
736 *Phytophthora capsici*. Mol. Plant Path. 13,329-337.
737 Lan, C.Z., Liu, P.Q., Li, B.J. et al. 2013. Development of a specific PCR assay for the rapid and
738 sensitive detection of *Phytophthora capsici* . Australasian Plant Pathol. 42, 379-384
739 https://doi.org/10.1007/s13313-012-0185-8.

740 Langarica-Fuentes, A., Zafar, U., Heyworth, A., Brown, T., Fox, G., Robson, G.D., 2014. Fungal
741 succession in an in vessel composting system characterized using 454 pyrosequencing.
742 FEMS Microbiol. Ecol. 88,296-308.
743 Lauber, C.L., Hamady, M., Knight, R. & Fierer, N., 2009. Pyrosequencing-based assessment of soil
744 pH as a predictor of soil bacterial community structure at the continental scale. Appl.
745 Environ. Microbiol. 75,5111-5120.
746 Lee, K.J., Kamala-Kannan, S., Sub, H.S., Seong, C.K., Lee, G.W., 2008. Biological control of
747 Phytophthora blight in red pepper (*Capsicum annuum* L.) using *Bacillus subtilis*. World J.
748 Microbiol. Biotechnol., 24,1139-1145.
749 Li, H., Cai, X., Gong, J., Xu, T., Ding, G. C., & Li, J., 2019. Long-term organic farming
750 manipulated rhizospheric microbiome and *Bacillus* antagonism against pepper blight
751 (*Phytophthora capsici*). Front. microbiol, 10, 342. doi:10.3389/fmicb.2019.00342.
752 Lombardi, N., Vitale, S., Turrà, D., Reverberi, M., Fanelli, C., Vinale, F., et al., 2018., Root
753 exudates of stressed plants stimulate and attract *Trichoderma* soil fungi. Mol. Plant-Microbe
754 Interact. 31, 982-994.
755 López-Mondèjar, R., Raidl, S., Ros, M., Pascual, .JA., 2010. Quantification of the biocontrol agent
756 *Trichoderma harzianum* with real-time TaqMan PCR and its potential extrapolation to the
757 hyphal biomass.; Bioresour. Technol. 101, 2888-2891.
758 Lorito, M., Woo, S. L., 2015b. “Discussion agronomic,” in *Principles of plant-microbe interactions*
759 (ed. Lugtenberg, B.), ed. Berlin: Springer International Publishing, 345-353, 10.1007/978-3-
760 319-08575-3_36.
761 Lorito, M., Woo, S.L., 2015a. “*Trichoderma:* a multi - purpose toll for integrated pest
762 management”, in *Principles of plant-microbe interactions* (ed. Lugtenberg, B.), ed. Berlin:
763 Springer International Publishing.
764 Lorito, M., Woo, S.L., Harman, G.E., Monte, E., 2010. Translational research on *Trichoderma*:
765 from ’omics to the field. Annu. Rev. Phytopathol. 48,395-417.
766 Magoc, T. and Salzberg, S.L., 2011. FLASH.: Fast length adjustment of short reads to improve
767 genome assemblies. Bioinfo. 27,2957-2963.
768 McKellar, M.E., Nelson, E.B., 2003. Compost-induced suppression of Pythium damping-off is
769 mediated by fattyacid-metabolizing seed-colonizing microbial communities. Appl. Environ.
770 Microbiol. 69,452-460.
771 Mendes, R., Garbeva, P., Raaijmakers, J.M., 2013. The rhizosphere microbiome: significance of
772 plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microb.
773 Rev. 37, 634-663.
774 Mota-Gutierrez J., Ferrocino, I., Rantsiou K., Cocolin L., 2019 Metataxonomic comparison between
775 internal transcribed spacer and 26S ribosomal large subunit (LSU) rDNA gene. Int. J. Food
776 Microbiol., 290, 132-140 .
777 Muchovej, J.J., Maffia, L.A., Muchovej, R.M.C., 1980. Effect of exchangeable soil aluminium and
778 alkaline calcium salts on the pathogenicity and growth of *Phytophthora capsici* from green
779 pepper. Phytopathol. 70, 1212-1214.
780 Naing, K.W., Anees, M., Nguyen X.H., Lee Y.S., Jeon, S.W., Kim S.J., Kim, M.H., Kim K.Y.,
781 2014. Biocontrol of late blight disease (*Phytophthora capsici*) of pepper and the plant
782 growth promotion by *Paenibacillus ehimensis* KWN38, J. Phytopathol1. 62, 367-376.
783 Nguyen, X.-H., Naing, K.-W., Lee, Y.-S., Tindwa, H., Lee, G.-H., Jeong, B.-K., Ro, H.-M., Kim,
784 S.-J., Jung, W.-J., Kim, K.-Y., 2012. Biocontrol potential of *Streptomyces griseus* H7602
785 against root rot disease (*Phytophthora capsici*) in pepper. Plant Pathol. J. 28, 282-289.
786 Nicol G. W., Leininger S., Schleper C., Prosser J. I., 2008. The influence of soil pH on the
787 diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria.
788 Environ. Microbiol. 10, 2966-2978.
789 Noble R., Coventry E., 2005. Suppression of soil-borne plant diseases with composts: a review.
790 Biocon. Sci. Technol.15, 3-20.

791 Nunez-Zofio, M., Larregla, S., Garbisu, C., 2011. Application of organic amendments followed by
792 soil plastic mulching reduces the incidence of *Phytophthora capsici* in pepper crops under
793 temperate climate. Crop Prot. 30, 1563-1572.
794 Olsen, S., Cole, C., Watanabe, F. and Dean, L., 1954. Estimation of available phosphorus in soils
795 by extraction with sodium bicarbonate. USDA Circular Nr 939. Washington, DC: US Gov.
796 Print. Office.
797 Padley, L.D., Jr. Kabelka, E.A., Roberts, P., French, R., 2008. Evaluation of *Cucurbita pepo*
798 accessions for crown rot resistance to isolates of *Phytophthora capsici*. Hort Sci. 43,1996-
799 1999.
800 Paris, H.S. and Maynar, D.N., 2008. *Cucurbita* spp., squash, pumpkin, gourds. In Janick, J. and
801 Paull, R.E eds. *The encyclopedia of fruits and nuts*. CAB International, Wallingford,
802 Oxforshire, United Kingdom, pp. 292-299.
803 Parra, G., Ristaino, J.B., 2001. Resistance to mefenoxam and metalaxyl among field isolates of
804 *Phytophthora capsici* causing Phytophthora blight of bell pepper. Plant Dis. 85,1069-1075.
805 Philippot, L., Raaijmakers, J. M., Lemanceau, P., van den Putten, W.H., 2013. Going back to the
806 roots: the microbial ecology of the rhizosphere. Nat. Rev. Microbiol. 11,789-799.
807 Ploetz, R.C., Heine, G., Haynes, J., Watson, M., 2002. An investigation of biological attributes that
808 may contribute to the importance of *Phytophthora capsici* as a vegetable pathogen in
809 Florida. Ann. Appl. Biol. 140, 61-67.
810 Postma, J., Schilder, M.T. & van Hoof, R.A., 2011. Indigenous populations of three closely related
811 *Lysobacter* spp. in agricultural soils using real-time PCR. Microb. Ecol. 62, 948-958.
812 Pugliese, M., Liu, B.P., Gullino, M.L. and Garibaldi, A., 2011. Microbial enrichment of compost
813 with biological control agents to enhance suppressiveness to four soil-borne diseases in
814 green house. J. Plant Dis. Protect. 118, 45-50.
815 Pugliese, M., Gilardi, G., Garibaldi, A., Gullino, M. L., 2015. Organic amendments and soil
816 suppressiveness: results with vegetable and ornamental crops. In: Organic amendments and
817 soil suppressiveness in plant disease management (Meghvansi M. K. and Varma A. coord.),
818 Soil Biology 46, Springer, 495-509.
819 Ros, M., Raut, I., Santisima-Trinidad, A.B., Pascual, J.A., 2016. Relationship of microbial
820 communities and suppressiveness of *Trichoderma* fortified composts for pepper seedlings
821 infected by *Phytophthora nicotianae*. PLoS One, 12(3), e0174069, doi:
822 10.1371/journal.pone.0174069.
823 Rotthauwe, J.H., Witzel, K.P., Liesack, W., 1997. The ammonia monooxygenase structural gene
824 *amoA* as a functional marker: molecular fine-scale analysis of natural ammonia oxidizing
825 populations. Appl. Environ. Microbiol. 63, 4704-4712.
826 Rousk, J., Bååth, E., Brookes, P.C., et al., 2010. Soil bacterial and fungal communities across a pH
827 gradient in an arable soil. ISME J. 4, 1340-1351.
828 Sang, M. K., Kim, J.G., Kim, K.D., 2010. Biocontrol activity and induction of systemic resistance
829 in pepper by compost water extracts against *Phytophthora capsici*. Phytopathol. 100, 774-
830 783.
831 Sang, M. K., Shrestha, A., Kim, D. Y., Park, K., Pak, C. H., & Kim, K. D., 2013. Biocontrol of
832 Phytophthora blight and anthracnose in pepper by sequentially selected antagonistic
833 rhizobacteria against *Phytophthora capsici*. Plant Phatol. J., 9, 154-167.
834 Sang, M.K., Chun, S. and Kim, K.D., 2008. Biological control of Phytophthora blight of pepper by
835 antagonistic rhizobacteria selected from a sequential screening procedure. Biol. Control, 46,
836 424-433.
837 Segarra, G., Avilés, M., Casanova, E., Borrero, C., Trillas, I., 2013. Effectiveness of biological
838 control of *Phytophthora capsici* in pepper by *Trichoderma asperellum* strain T34.
839 Phytopathol. Mediterr. 52, 77-83.

840 Segarra, G., Casanova, E,. Bellido, D,. Odena, M.A,. Oliveira, E and Trillas, I., 2007. Proteome,
841 salicylic acid, and jasmonic acid changes in cucumber plants inoculated with *Trichoderma*
842 *asperellum* strain T34. Proteomics 7, 3943-3952.
843 Sid, A., Ezziyyani, M., Egea-Gilabert, C., Candela, M., 2003. Selecting bacterial strains for use in
844 the biocontrol of diseases caused by *Phytophthora capsici* and *Alternaria alternat*a in sweet
845 pepper plants, Biol. Plant. 47, 569-574.
846 Soil Survey Staff, 2010. Keys to Soil Taxonomy, 11th ed. USDA-Natural Resources Conservation
847 Service, Washington, DC.
848 Spadaro, D., Gullino, M.L., 2005. Improving the efficacy of biocontrol agents against soil-borne
849 pathogens. Crop Prot. 24, 601-613.
850 Steger, K., Jarvis, A., Smars, S., Sundh, I., 2003. Comparison of signature lipid methods to
851 determine microbial community structure in compost. J. Microbiol. Methods., 55, 371-382.
852 PMID: 14529958.
853 Sutherland, E.D., Papavizas, G.C., 1991. Evaluation of oospore hyperparasites for the control of
854 Phytophthora crown rot of pepper. J. Phytopathol. 131, 33-39.
855 Sy, O., Steiner, R., Bosland, P.W., 2008. Recombinant inbred line differential identifies race-
856 specific resistance to Phytophthora root rot in *Capsicum annum*. Phytopathol. 98, 867-870.
857 Tamietti, G., Valentino, D., 2001. Physiological characterisation of a population of *Phytophthora*
858 *capsici* Leon. from Northern Italy. J. Plant Path. 83, 199-205.
859 Tandon, A., Fatima, T., Gautam, A., Yadav, U., Srivastava, S., Singh, P.C., 2018. Effect of
860 *Trichoderma koningiopsis* on Chickpea rhizosphere activities under different fertilization
861 regimes. Open J. Soil Sc., 8, 261-275.
862 Thampi, A., Bhai, R.S., 2017. Rhizosphere actinobacteria for combating *Phytophthora capsici* and
863 *Sclerotium rolfsii*, the major soil borne pathogens of black pepper (*Piper nigrum* L.). Biol.
864 Control 109:1-13. doi:https://doi.org/10.1016/j.biocontrol.2017.03.006.
865 Thébault, E., Fontaine, C., 2010. Stability of ecological communities and the architecture of
866 mutualistic and trophic networks. Science. 329, 853-856.
867 Verma, M., Brar, S.K., Tyagi, R.D., Surampalli, R.Y., Valéro, J.R., 2007. Antagonistic fungi,
868 *Trichoderma* spp.: Panoply of biological control. Biochem. Eng. J. 37,1-20.
869 Vinale, F., Sivasithamparam, K., Ghisalberti, E.L., Marra, R., Barbetti, M.J., Li, H., et al., 2008. A
870 novel role of *Trichoderma* secondary metabolites in the interaction with plants. Physiol.
871 Mol. Plant. Pathol. 72, 80-86.
872 Wallenstein, M.D., 2017. Managing and manipulating the rhizosphere microbiome for plant health:
873 a system approach. Rhizosphere, 3, 230-232.
874 Wang, Z., Johnston, P.R., Takamatsu, S., Spatafora, J.W., Hibbett, D.S., 2006. Toward a
875 phylogenetic classification of the Leotiomycetes based on rDNA data. Mycologia, 98, 1065-
876 1075.
877 Wei, Z., Yang, X., Yin, S., Shen, Q., Ran, W., Xu, Y., 2011. Efficacy of *Bacillus*-fortified organic
878 fertiliser in controlling bacterial wilt of tomato in the field, Appl. Soil Ecol. 48, 152-159.
879 Woo, S.L., Ruocco, M., Vinale, F., Nigro, M., Marra, R., Lombardi, N., 2014. *Trichoderma* based
880 products and their widespread use in agriculture. Open Mycol. J. 8,71-126.
881 Woo, S.L., Scala, F., Ruocco, M., Lorito, M., 2006. The molecular biology of the interactions
882 between *Trichoderma* spp., phytopathogenic fungi, and plants. Phytopathol. 96,181-185.
883 Yang, M.M., Xu, L.P., Xue, Q.Y., Yang, J.H., Xu, Q., Liu, H.X., Guo, J.H., 2012. Screening
884 potential bacterial biocontrol agents towards *Phytophthora capsici* in pepper, Eur. J. Plant
885 Pathol. 1-10.
886 Yuan, J., Raza, W., Shen, Q., Huang, Q., 2012. Antifungal activity of *Bacillus amyloliquefaciens*
887 NJN-6 volatile compounds against *Fusarium oxysporum* f. sp *cubense*. Appl. Environ.
888 Microbiol. 78, 5942-5944.

889 Zhang, S., White, T.L., Martinez, M.C., McInroy, J.A., Kloepper, J.W., Klassen, W., 2010.
890 Evaluation of plant growth-promoting rhizobacteria for control of Phytophthora blight on
891 squash under greenhouse conditions. Biol Control, 53, 129-135.
892

893

894

895

896 .

897

898

899

900

901

902

903

904

905

906

907

908

909

910

911

912

913

914

915

916 **Figure Captions**

917 Figure 1 Boxplots of the α-diversity measures of microbiota (Panel A) and mycobiota (Panel B) of
918 the soils. Boxplot display Chao1 index, estimating the number of different taxa, Shannon diversity
919 index evaluating the taxa richness as well as number of OTUs observed. The bars are color-coded
920 according to the site and the sampling year. Individual points and brackets represent the richness
921 estimate and the theoretical standard error range, respectively.

922

923 Figure 2 Principal coordinate analysis of the Unweighted UniFrac distances of the 16S rRNA gene
924 sequence data. The samples are color-coded according to the site and the sampling year