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Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1562049> since 2017-07-03T18:26:02Z

Published version:

DOI:10.1016/j.funbio.2016.03.008

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Influence of plant genotype on the cultivable fungi associated to tomato rhizosphere and roots in different soils

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Running Head: Root and rhizosphere mycobiota of tomato

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Abstract

Rhizosphere and root-associated microbiota are crucial in determining plant health and in increasing productivity of agricultural crops. To date, research has mainly focused on the bacterial dimension of the microbiota. However, fungi play a key role in soil ecosystems, being involved in symbiosis, plant pathogenicity, or biocontrol. Consequently, interest in the mycobiota is rapidly increasing. In this work, we examined the effect of plant genotype, soil, and of the pathogen *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) on the cultivable component of rhizosphere and root-associated mycobiota of tomato plants. Resistant (Heinz 1706) and susceptible (Moneymaker) varieties were cultivated on two soils diverse for their history, geographical origin, physical and chemical characteristics, (identified as A and B throughout the work), under glasshouse conditions. Isolated fungi were identified by morphological and molecular approaches. The lower diversity was retrieved from the combination soil A/Moneymaker, where *Fusarium*, *Trichoderma*, and *Penicillium* were the most represented genera. Differences were found when comparing the rhizosphere to the roots, which in general displayed a lower number of species. The structure of the cultivable mycobiota was significantly affected by the soil type in the rhizosphere as well as by the plant genotype within the roots (NPERMANOVA, $p < 0.05$). The addition of *Fol* to Heinz 1706 changed the community structure, particularly in soil A, where *Penicillium* spp. and *Fusarium* spp. were the dominant responding fungi. Overall, the results indicated that i) soil type and plant genotype affect the fungal communities; ii) plant roots select few species from the rhizosphere; and iii) the fungal community structure is influenced by the pathogen.

Keywords: Mycobiota, Fusarium wilt, Plant Genotype, Soil Type

1. Introduction

In plants, a microbiota is an interactive microorganism community associated with the plant rhizosphere and roots, which plays a crucial role in influencing plant health (Abd-El salam et al. 2010; Mendes et al. 2011). Likewise, the microbial community is affected by both plant and soil type; specific members of the microbiota are stimulated or repressed by chemical exudates released in the rhizosphere, the root-surrounding soil region (Berendsen et al. 2012).

Vegetable and ornamental crops are often attacked by several soilborne pathogens, resulting in economic losses. Tomato (*Lycopersicum esculentum*) is a popular and economically relevant culture and has been proposed as a model for studying plant-pathogens interactions, since its productivity can be limited by a number of diseases caused by viruses, bacteria and fungi (Arie et al. 2007). One of the major soilborne pathogens that endangers tomato crops worldwide is *Fusarium oxysporum*, the causal agent of Fusarium wilt, which is capable of affecting a variety of crops species. *F. oxysporum* has been subdivided in over 120 morphologically undistinguishable *formae speciales*, depending on the host plant (Michielse and Rep 2009), further classified into physiological races on the basis of cultivar specificity (Di Pietro et al. 2003). To date, management of wilt disease relies mainly on soil disinfestation and use of resistant cultivars. However, several compounds have been banned or limited in their use. As for the use of resistant cultivars, new more virulent races frequently arise to overcome the host resistance (Kinkel et al. 2011). Therefore, due to the possible alternatives in disease control (Fravel et al. 2003; Mazzola 2002, 2004), the search for potential biocontrol agents is intensifying.

The microbial community *in toto* (bacteria, fungi, pseudofungi and protozoa) is considered to be crucial for plant protection and novel discoveries are necessary to improve crop quality and yield. As supported by a number of studies, several factors including the

plant species, the plant genotype and the soil type are capable of shaping the rhizosphere microbiota (Hardoim et al. 2011; Inceoglu et al. 2012; Philippot et al. 2013). Considering the fact that plant resistance represents one of the strategies to overcome vascular diseases, several studies have been conducted on a number of crops in order to clarify the effects of resistant and susceptible cultivars on microbial communities (An et al. 2011; Azad et al. 1987; Nallanchakravarthula et al. 2014; Yao and Wu 2010). The soil microbial community has been demonstrated to be significantly affected by the plant genotype, indicating a role of the rhizosphere microorganisms in conferring resistance to pathogens (An et al. 2011; Inceoglu et al. 2012; Nallanchakravarthula et al. 2014).

Along with the rhizosphere microorganisms, the so-called “endophytes” which are associated to the plant tissues, are a relevant component of the root microbiome. The endophytic community, as the rhizospheric community, is important for plant growth and is influenced by plant and soil factors, and microbial features responsible for the survival of endophytes within the roots (Gaiero et al. 2013; Turner et al. 2013).

Understanding the rules that drive formation of a plant microbiome and identifying its components is a crucial point to increase productivity and reduce pathogen attacks. To date, several studies have mainly focused on the bacterial microbiota (Bulgarelli et al. 2013; Chaparro et al. 2014; Inceoglu et al. 2012; Spence et al. 2014; Turner et al. 2013), while a void has still to be filled on the fungal community and its function, although research on this topic is rapidly increasing (Nallanchakravarthula et al. 2014; Nam et al. 2015; Yao and Wu 2010).

Tomato is known to differentially respond to beneficial (Salvioli et al. 2012), pathogenic and biocontrol fungi (Spadaro and Gullino 2005) and genotypes with different features provide an unprecedented model to investigate the network of interactions taking place belowground. With the present work, we intended to shed a light on the cultivable

component of the mycobiota associated to tomato plant, clarifying how the soil and the plant genotype can determine its shaping. In addition, we aimed to assess whether the presence of a fungal pathogen could modify the structure of the rhizosphere and root associated fungal community. Finally, the availability of fungal cultures (both from rhizosphere and roots) would offer valuable tools to investigate the functionality of the fungal communities with the intent of reconstructing specific tomato microbiomes; to this aim, cultivable fungi only were considered in this work.

2. Materials and methods

2.1. Plant cultivars, experimental soils and plant growth

Two cultivars of tomato and two different soils were used in this study. The cultivars Heinz 1706 and Moneymaker, were selected as resistant (R) and susceptible (S) to *Fusarium oxysporum* f. sp. *lycopersici*, respectively (Huang and Lindhout 1997; Ozminkowski 2004). Two soils, A and B, were collected in Northern Italy and chosen on the basis of their different history, physical and chemical characteristics which were determined by AgroBio Lab (Rutigliano, Italy) with accredited methods for pH, structure, organic carbon, total nitrogen, mineral composition, and conductivity (Table 1). Soil A was cultivated with vegetables since 1980 while soil B was taken from a field where wheat was cultivated for 15 years and later the soil was set aside for ten years (no crops were grown).

Tomato seeds of both cultivars were sown in plug trays (80 plugs/tray) containing peat-perlite substrate and were watered daily. Following, three 14 days old tomato seedlings were transplanted in 2 L pots containing either soil A or soil B. Three pots were prepared for each treatment. Plants were maintained for 4 weeks under glasshouse conditions (temperature ranging between 26°C and 28°C; automatic watering and shading).

In order to evaluate the influence of a soilborne pathogen on the mycobiota of the resistant cultivar, *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) was inoculated in both soils. Prior to seedling transplant the two soils were mixed with *Fol* in form of talc powder (Srinivasan et al. 2009) at the final rate of 3×10^4 chlamydospores mL⁻¹ of soil.

2.2. Isolation and identification of cultivable fungi

2.2.1. Sample collection

Following careful removal of the aboveground plant, rhizospheric soils derived from Heinz 1706 and Moneymaker tomato plants cultivated in soil A and B, were treated as described by Lundberg et al. (Lundberg et al. 2012). Briefly, loose soil was removed from the roots by gently shaking and patting with sterile gloves. Roots were placed in sterile 50 mL tubes containing 25 mL phosphate buffer and vortexed to release most of the rhizospheric soil. To remove large debris, the turbid solution was filtered into a new 50 mL tube and centrifuged for 15 min at 3,500 g. The supernatant was discarded and the loose pellets containing microorganisms was resuspended and transferred to 1.5 ml tubes. Following centrifugation at 10,000 g for 5 min, pellets were processed further, as described in the next section. In parallel, the root systems were transferred to clean sterile tubes, and serially washed until the buffer was clear after vortexing.

2.2.2. Isolation of fungi from the rhizosphere

Rhizosphere samples were analysed by soil dilution plate method on two agar media, as follows. A phosphate buffer dilution of 10^{-4} was prepared from about 1 g of fresh soil obtained from roots serial washing. One mL of the final dilution was mixed with 30 ml of Malt Extract Agar (MEA) or Komada's medium (selective for *Fusarium* spp.), supplemented with antibiotics (streptomycin, 0.015 g L⁻¹; chloramphenicol, 0.05 g L⁻¹) and placed in 15 cm diameter Petri dishes.

For each plant and medium, three replicates were performed. Following incubation at 24°C in the dark for 7-10 days, colony forming units (CFU) were counted by visual observation and isolated in pure culture. The fungal load (CFU per g of dry weight) was then calculated both for the total mycoflora and for each species or morphotype.

2.2.3. Isolation of roots associated fungi

For the isolation of endophytic fungi, ten 0.5 cm specimens for each cleaned plant root, were sonicated in sterile distilled water at low intensity five times for 30 seconds and placed in 15 cm Petri dishes containing MEA or Komada. Three replicates were performed for each plant and medium. Samples were incubated in the dark at 24°C and colony growth was monitored over time up to 30 days. Colonies isolation and count were accomplished following the methods described above.

2.2.4. Morphological and molecular identification

Morphological identification of each strain was achieved according to the relevant taxonomic keys (Domsch et al. 1980; Kiffer and Morelet 1997; von Arx 1981) and confirmed by sequencing the appropriate DNA region (ITS, α -actin, β -tubulin). Genomic DNA of all strains was extracted from about 100 mg of mycelium scraped from PDA Petri dishes using the NucleoSpin kit (Macherey Nagel GmbH, Duren, DE, USA), according to the manufacturer's instructions. The quality and quantity of DNA samples was measured with the ND-1000 Spectrophotometer NanoDropH (Thermo Scientific, Wilmington, Germany). DNA extracts were stored at -20°C. The ITS sequences were amplified using the primer pair ITS1/ITS4 (White et al. 1990). For those strains morphologically identified as *Aspergillus* spp. and *Penicillium* spp., amplification of the β -tubulin gene was performed using primers Bt2a/Bt2b (Geiser et al. 1998; Glass and Donaldson 1995; Samson et al. 2004), while molecular identification of species belonging to the genus *Cladosporium* spp. was inferred through the analysis of the α -actin gene using the primer pair ACT-

512F/ACT-783R (Carbone and Kohn 1999). Reaction mixtures consisted of 30 ng genomic DNA, 1 μ M each primer, 1 U Taq DNA Polymerase (Qiagen, Chatsworth, CA, USA), 10x buffer, and 200 μ M each dNTP. DNA amplifications were performed using a T-Gradient thermal cycler (Biometra, Göttingen, Germany) with the following profile: 95°C for 5 min; 35 cycles: 95°C for 40 sec, 55°C (58°C for Bt2a/Bt2b) for 45 sec, 72°C for 50 sec; 72°C for 8 min. PCR products were purified and sequenced at Macrogen Europe Laboratory (Amsterdam, The Netherlands).

To confirm pathogen inoculation, genomic DNA of all *Fusarium oxysporum* isolates was subjected to microsatellite screening by using the core sequence of the microsatellite M-13 as a primer (Abd-Elsalam et al. 2010; Asran-Amal et al. 2005). Amplicons were separated on 1.5% agarose gel stained with 5 μ L 100 mL⁻¹ ethidium bromide and a GelPilot 1 kb plus DNA Ladder was used; images were acquired with a Gel Doc 1000 System (Bio-Rad, Hercules, CA, USA) and fingerprints were analysed against the positive control used for inoculum preparation (*Fol*) using Bionumerics 7.1 software.

Representative strains of each species isolated in pure culture during this work are preserved at Mycotheca Universitatis Taurinensis (MUT). The Accession numbers of the sequences deposited in GenBank are: **KR709174-KR709205, KR856498-KR856506, KT013225-KT013243, KT030798.**

2.3. Disease suppression assay

In light of the findings that the organisms isolated included species, which may act as biological control agents, the two soils were tested for disease suppression potential. Steamed peat (30 min at 70 °C) served as control. Following soil inoculation with *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*), five 14 days old tomato seedlings of both cultivars were transplanted in 2 L pots. *Fol* was supplied in a talc powder form at a concentration of 3×10^4 chlamydospores mL⁻¹ of soil, as previously described. Four pots per treatment were set

up. Plants were maintained under glasshouse conditions with temperature ranging between 26°C and 28°C. Symptoms started to be visible 14 days after artificial inoculation. Disease development was monitored weekly, and a disease index ranging from 0 to 100 was assigned throughout the experiments (0 = healthy plant; 25 = slight leaf chlorosis; 50 = severe leaf chlorosis, growth reduction and initial symptoms of wilting; 75 = severe wilting symptoms, leaf chlorosis and strong growth reduction; 100 = dead plant). Symptomatic plants showed brown or black streaks in the vascular system. The final disease index was evaluated 30 days after transplant, and a stem dissection confirmed the presence of *Fol*, revealed by discoloration of the vascular system. The experiment was repeated three times.

2.4. Statistical analysis

NPMANOVA and Biodiversity analyses were performed using PAST (PAleontological STatistics) software for data analysis in ecology (Huang et al. 2013) available on the Web (<http://folk.uio.no/ohammer/past/>).

Statistical significance on the total fungal load (CFU g⁻¹ dwt) was inferred by applying the analysis of variance (ANOVA), Bonferroni post hoc test (p<0.05), using GraphPad Prism 5 software. In order to avoid sampling size effects, the number of CFU per sample was normalized by randomly subsampling to the lowest number of CFU among samples; the relative abundance of species was used for NPMANOVA analysis using Bray-Curtis dissimilarity indices. Subsampling was achieved by means of *rrarefy* function in the R package *vegan* (Oksanen et al. 2013). Biodiversity of fungal communities was compared in four conditions (SA, Susceptible cultivar/soil A; SB, Susceptible cultivar/soil B; RA, Resistant cultivar/soil A; RB, Resistant cultivar/soil B) by applying the Shannon (H, which incorporates species richness and species evenness) and Simpson (D, which incorporates species richness and abundance) diversity indices, which were statistically compared (Mann-Whitney test; p<0.05).

3. Results

3.1. Soils features

Soil A and soil B were collected in Northern Italy (Liguria and Piedmont respectively) and analysed for the respective chemical features. Both soils presented a similar soil texture, with a high proportion of sand, followed by clay and silt. A sandy clay loam texture was observed in both cases. The pH was similar, although slightly higher in soil B. Organic carbon was much higher in soil A while the total nitrogen was similar between the two soils. Soil A was characterized by a high conductivity and a high presence of minerals, including potassium, sodium and iron. Finally, magnesium was high in both soils (Table 1).

3.2. Direct counts of fungal communities in soil and roots

The total fungal load for cultivable rhizospheric fungi ranged from 3.68×10^5 to 6.50×10^6 CFU g⁻¹ dwt, with significant differences among the samples (Fig. 1A). The highest fungal load was found in the soil A cultivated with the susceptible cultivar (6.50×10^6 CFU g⁻¹ dwt), while a significant reduction was present in soil B for both cultivars (3.68×10^5 CFU g⁻¹ dwt and 6.48×10^5 CFU g⁻¹ dwt for susceptible and resistant, respectively). A significant difference between the two cultivars was observed only in soil A (6.50×10^6 CFU g⁻¹ dwt and 1.48×10^6 CFU g⁻¹ dwt for susceptible and resistant, respectively). As for the endophytic fungi, the fungal load changed significantly only when the resistant cultivar was grown on the soil B (Fig. 1B).

A total of 84 fungal entities, belonging to 40 genera, were identified from soil of tomato plants. The highest number of species was isolated from the rhizosphere (81 species belonging to 39 genera). As for the roots, 24 species belonging to 15 genera (“endophytic fungi” throughout the work) were detected; of these, 22 were in common with the rhizosphere, while *Myrothecium verrucaria* and *Setophoma terrestris* were exclusively

isolated from roots. The majority of the species belonged to the Ascomycota, whereas one single Basidiomycota (*Irpex lacteus*) was recovered from the rhizosphere of soil B. Seven Zygomycota were also detected (Table S1).

The statistical analysis of the normalized data relative to the fungal abundance in the rhizosphere, showed that the soil had a strong effect on the variance (NPERMANOVA; $p < 0.05$) while in the roots both soil and genotype, and the combination of these two factors, influenced significantly the structure of the mycobiota (Table 2).

Diversity of fungal communities was also compared. The Shannon biodiversity index was significantly lower ($p < 0.05$) when soil A was cultivated with Moneymaker for both the rhizospheric and endophytic species (Table 3). Moreover, a significant higher diversity was detected in the resistant cultivar/soil A for the rhizosphere and in the susceptible cultivar/soil B within the roots. The genera with the highest load in the rhizosphere were *Fusarium*, *Gibellulopsis*, *Penicillium*, *Phoma*, *Pyrenochaetopsis*, *Sarocladium*, and *Trichoderma*, whereas in the roots were *Fusarium* and *Trichoderma*. The genera with the highest load in soil A were *Fusarium* spp., *Phoma* spp., *Pyrenochaetopsis decipiens*, *Sarocladium strictum*, and *Trichoderma* spp. , while in soil B were *Trichoderma* spp., *Penicillium* spp., *Sarocladium strictum* and *Fusarium* spp. (Fig. 2 A & B, Table S1).

As shown in the Venn diagrams (Fig. S1), four rhizospheric species (*Trichoderma harzianum*, *Sarocladium strictum*, *Trichoderma longibrachiatum*, and *Penicillium carneum*) were common to all treatments (SA, SB, RA, RB). In soil A, the highest number of both total (34 vs 19) and exclusive (15 vs 5) species was recorded on the resistant cultivar, while in soil B the number of total (24 vs 26) and exclusive (10 vs 10) species was almost identical between the resistant and susceptible cultivar. Finally, the number of species in common between the two cultivars was almost identical in both soils (10 and 11, respectively). When considering the endophytic fungi, none of the species isolated was

shared among the four treatments. Interestingly, *Trichoderma harzianum* was the only organism common to three treatments (SA, RA, RB) (Fig. S1B). Furthermore, the percentage of species exclusive for one of any treatment was higher in the roots (86% exclusive vs 14% shared) while, in the rhizosphere, 59% of the species were unique and 41% were common to at least two conditions (Table S1).

3.3. Effect of pathogen addition on the fungal community

Fingerprints images obtained from M-13 microsatellite amplification of all *Fusarium oxysporum* isolates were analysed against the positive control used for inoculum preparation (*Fol*). *Fol* was re-isolated from the rhizosphere and roots of tomato planted on both soils (Fig. 3).

The addition of the pathogen generally did not have a significant influence on the load and fungal diversity in the rhizosphere in both soils (Fig. 4A; Table 3). However, a deeper analysis revealed that, following the inoculation of the pathogen, soil A responded with a significant increment of *Fusarium* spp., *Penicillium* spp., and *Trichoderma* spp. in the rhizosphere (Fig. 5A) and of *Fusarium* spp. and *Zygomycetes* spp. in the roots (Fig. 5B). As for soil B, the number of total CFU increased only in endophytic fungi (Fig. 4B). In addition, few species (*Acremonium crotonigenum*, *Aspergillus fumigatus*, *Cladosporium oxysporum*, *Doratomyces stemonitis*, *Penicillium griseofulvum*, *Penicillium spinulosporum*) were detected only in the presence of *Fol*, suggesting a change in the mycoflora composition.

3.4. Disease suppression assay

Considering the results described above, a disease suppression assay was performed in order to evaluate whether the species retrieved in soil A could reduce the incidence of *Fusarium* wilt. The assessment of a disease index showed that only the susceptible cultivar

(Moneymaker) developed wilt disease, which showed a reduction both in soil A (2.8 %) and soil B (27.1 %) in comparison to steamed peat (57.2%) which was used as a control. No disease symptoms appeared in the resistant cultivar Heinz 1706, as expected. Due to the high variability however, the difference between the two soils was not significant, even though a clear trend was observed.

5. Discussion

Taking in consideration multiple parameters (plant genotype, soil, pathogen presence) we demonstrate that soil is the major driving force in shaping the cultivable mycobiota, where *Fusarium*, *Penicillium*, *Sarocladium* and *Trichoderma* genera resulted dominant. Alternatively, a genotype effect and a lower fungal diversity were found among fungi with an endophytic profile different from the more diverse rhizospheric fungi.

In terms of quantitative evaluation, a significantly higher fungal load for the susceptible cultivar was measured in the rhizosphere of soil A, although the lower diversity observed could be due to a soil/cultivar synergic effect. Our results are similar to those recently reported by Nallanchakravarthula et al. (2014), who assessed the influence of soil type and cultivars on the rhizosphere and root mycobiota of strawberry, demonstrating a stronger effect of soil respect to the plant genotype. It could reasonably be argued that a variation in fungal diversity may be due to different physical-chemical properties of the soils, although this can not completely explain the differences observed in soil A between the susceptible (>CFU) and resistant cultivars (Fig. 1A). This aspect was taken into consideration in determining the structure of fungal communities in suppressive and conducive soils to *Rhizoctonia solani*, clarifying that it was not as crucial as the one associated to suppression abilities (Penton et al. 2014), thus supporting the hypothesis that the difference observed is due to the mycotic community specific for each soil.

The effect of plant genotype on fungal population was not significant when soil B was evaluated (Fig. 1A), in contrast with the observations on the rhizosphere of different cultivars of cucumber, where a higher number of CFU was associated with the cultivar susceptible to Fusarium wilt (Yao and Wu 2010). The opposite behaviour of the pair Moneymaker/Heinz 1706 which appears to be clear in soil A, vanished in soil B suggesting a synergism between the factors soil and genotype.

Regarding the endophytes, significant differences were detected in the resistant cultivar when grown on soil A(>CFU) or soil B (<CFU) (Fig 1B). This may be explained considering the fact that in soil A a wider fungal population might penetrate the roots of the resistant cultivar for the following reasons: (i) the abundance of the species in soil A is slightly higher, (ii) some of these species may be involved in biological control processes, (iii) none of the species present in the rhizosphere compromise the resistant cultivar; consequently the root colonization would not affect the plant health and the resistance properties. In addition, some species appear to be recruited by the resistant genotype cultivated on soil B (i.e. *Penicillium* spp. and *Trichoderma* spp.). A soil effect is evident, since a similar trend, although not significant, can be noticed in the susceptible cultivar, probably as a consequence of a higher root colonization due to poor resistant mechanisms of the plant.

When a random subsampling was applied to normalize the number of CFU with the intent of avoiding sampling size artifacts, a genotype effect on the fungal community was evident only within the roots, while a dominant role was played in the rhizosphere by the soil. This may be explained by hypothesizing that in the rhizosphere the difference inferred by the soil is prevalent to such an extent that the genotype factor appears to be trivial. Besides, it has to be considered that roots are the first plant organs to come physically in contact with the microbiome belowground, thus disclosing the importance of

the plant genotype in selecting different microorganisms (Lakshmanan et al. 2014). To this respect, diverse genetic profiles of *Arabidopsis thaliana* have been proved to influence the production and secretion of phytohormones, which in turn modulate the assemblage of the endophytic bacterial community (Lebeis et al. 2015). Species of *Penicillium* (e.g. *P. canescens* and *P. rubens*) and *Paecilomyces marquandii* have been found almost exclusively in the rhizosphere of the susceptible plants grown in soil A. These species, together with others belonging to the genera *Chaetomium*, *Gliocladium*, *Penicillium*, *Paecilomyces*, *Sporothrix* and *Trichoderma* are known for their biocontrol properties (Paulitz and Belanger 2001; Punja and Utkhede 2003). Thus, the genotype of tomato plants may be important for the selection of a pool of useful organisms naturally present in a soil. This hypothesis may explain the lower diversity observed in this condition. Interestingly, the addition of the pathogen *Fol* to soil A, evaluated on the resistant cultivar only, is associated to a significant increase in CFU of *Fusarium* spp., *Penicillium* spp. and *Trichoderma* spp. in the rhizosphere, as reported by a number of studies (Berg et al. 2005; Rivera et al. 2009). The ability of a soil to contain a disease is usually ascribed to the effects of a number of microorganisms and the three genera retrieved include species largely recognised as biocontrol agents. Several studies attest the antagonistic activity of species of *Penicillium* spp. (e.g. *P. canescens*, *P. funiculosum*, *P. oxalicum* and *P. rubens* (Chen et al. 2006; Larena et al. 2003; Nicoletti et al. 2007; Radhakrishnan et al. 2013; Sabuquillo et al. 2006), *Trichoderma* spp. (Balasubramanian et al. 2014; Dubey et al. 2007; Nel et al. 2006b) and non pathogenic *Fusarium* spp. (Aime et al. 2013; Fuchs et al. 1999; Nel et al. 2006a, b) against *Fusarium* wilt. The suppression of a soilborne disease is generally accomplished through the secretion of plant growth promoting metabolites, such as indole acetic acid (Radhakrishnan et al. 2013), production of antibiotics, competition for nutrients, mycoparasitism and induction of plant defence reactions (Vos et al. 2014). Presumably, the

presence of *Fol* allows the plant to draw in its vicinity those beneficial fungal species naturally present in a soil which are capable of conferring protection. For instance, *P. canescens*, known for the production of fungitoxic secondary metabolites strongly increases its load in the presence of *Fol* (Nicoletti et al. 2007). Therefore, a relation between a pathogenic species and potential biocontrol taxa should be considered, as also proposed by Vujanovic et al. (2007), who conducted a survey on fungal species associated with black spruce trees.

A different situation occurred in the roots: *Penicillium* spp. and *Trichoderma* spp. were not recovered, contrary to *Fusarium* spp. (which can be only partly ascribed to the presence of the inoculum) and to the fast growing Zygomycetes, which can dominate an environment free of other competitors. Among the *F. oxysporum* strains isolated from the roots of plants cultivated on soil A, beside *Fol*, a number of strains with different genetic profiles, which may be both pathogenic and/or antagonists, were detected. Non pathogenic as well as pathogenic *F. oxysporum* can colonize tomato roots (Bao and Lazarovits 2001) as demonstrated for the well-studied biocontrol strain *F. oxysporum* 47 (Fo47), whose artificial root inoculation has been proved to increase the expression of genes encoding extracellular proteins potentially involved in the salicylic acid-dependent plant defence pathway (Aime et al. 2013). Finally, a change in the composition of the fungal community can be asserted, since few species are present or absent only in this condition.

In light of these findings, it was reasonable to assess whether soil A had the potential to prevent Fusarium wilt in tomato. To this aim, a disease suppression assay was performed, and a decrease of the disease incidence was recorded in both soils in comparison to the steamed peat. Every natural soil possesses the ability to counteract a disease to different degrees, depending on the biotic components (Mazzola 2004). However, the disease index in soil A was slightly lower than soil B, although due to the high variability

observed a statistical significance was not inferred. The observed disease reduction may be due to the presence of antagonistic bacteria and/or fungi; however, clarifying to which extent these organisms contribute to the feature displayed by soil A, was not the purpose of this work and further detailed studies will be necessary.

In conclusion, this work shows a major driving force of the soil type in shaping the rhizosphere mycobiota in tomato plants, while a significant role of the genotype was additionally found within the roots. Future studies will broaden the number of tested soils in order to extrapolate common features underlying soil ability to control a disease. In addition, the presence of the soilborne pathogen *Fol* is remarkable, since genera that may include agents of biocontrol positively respond to its presence particularly in the soil A. However, it must be considered that only cultivable fungi were contemplated, which could not completely account for the reported observations; a combined approach merging metagenomics and culturomics studies could unfold the entire scenario, as many cultured species fail to be identified by PCR-based methods only (Gouba et al. 2013, 2014).

Acknowledgements

This work was funded by MYCOPLANT project, Compagnia di San Paolo - Call 3 Strategic Research grants). Profs. Paola Bonfante and Mariangela Girlanda are acknowledged for support and useful suggestions throughout the work.

References

Abd-Elsalam KA, Almohimeed I, Moslem MA, Bahkali AH, 2010. M13-microsatellite PCR and rDNA sequence markers for identification of *Trichoderma* (Hypocreaceae) species in Saudi Arabian soil. *Genetics and Molecular Research* **9**, 2016-2024.

417 Aime S, Alabouvette C, Steinberg C, Olivain C, 2013. The Endophytic Strain *Fusarium*
 418 *oxysporum* Fo47: A Good Candidate for Priming the Defense Responses in Tomato Roots.
 419 *Molecular Plant-Microbe Interactions* **26**, 918-926.

420 An M, Zhou X, Wu F, Ma Y, Yang P, 2011. Rhizosphere soil microorganism populations
 421 and community structures of different watermelon cultivars with differing resistance to
 422 *Fusarium oxysporum* f. sp. *niveum*. *Can J Microbiol* **57**, 355-365.

423 Arie T, Takahashi H, Kodama M, Teraoka T, 2007. Tomato as a model plant for plant-
 424 pathogen interactions. *Plant Biotechnology*, 135-147.

425 Asran-Amal A, Abd-Elsalam KA, Omar MR, Aly AA, 2005. Antagonistic potential of
 426 *Trichoderma* spp. against *Rhizoctonia solani* and use of M13 microsatellite-primed PCR to
 427 evaluate the antagonist genetic variation. *Zeitschrift Fur Pflanzenkrankheiten Und*
 428 *Pflanzenschutz-Journal of Plant Diseases and Protection* **112**, 550-561.

429 Azad H, Davis J, Schnathorst W, Kado C, 1987. Influence of verticillium wilt resistant and
 430 susceptible potato genotypes on populations of antagonistic rhizosphere and rhizoplane
 431 bacteria and free nitrogen fixers. *Applied Microbiology and Biotechnology* **26**, 99-104.

432 Balasubramanian N, Priya VT, Shanmugaiah V, Lalithakumari D, 2014. Effect of
 433 improved *Trichoderma* fusants and their parent strains in control of sheath blight of rice
 434 and wilt of tomato. *Journal of Plant Diseases and Protection* **121**, 71-78.

435 Bao JR, Lazarovits G, 2001. Differential colonization of tomato roots by nonpathogenic
 436 and pathogenic *Fusarium oxysporum* strains may influence *Fusarium* wilt control.
 437 *Phytopathology* **91**, 449-456.

438 Berendsen RL, Pieterse CMJ, Bakker PAHM, 2012. The rhizosphere microbiome and plant
 439 health. *Trends in Plant Science* **17**, 478-486.

440 Berg G, Zachow C, Lottmann J, Gotz M, Costa R, Smalla K, 2005. Impact of plant species
 441 and site on rhizosphere-associated fungi antagonistic to *Verticillium dahliae* kleb. *Applied*
 442 *and Environmental Microbiology* **71**, 4203-4213.

443 Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P, 2013.
 444 Structure and Functions of the Bacterial Microbiota of Plants. *Annual Review of Plant*
 445 *Biology*, Vol 64 **64**, 807-838.

446 Carbone I, Kohn LM, 1999. A method for designing primer sets for speciation studies in
 447 filamentous ascomycetes. *Mycologia* **91**, 553-556.

448 Chaparro JM, Badri DV, Vivanco JM, 2014. Rhizosphere microbiome assemblage is
 449 affected by plant development. *ISME Journal* **8**, 790-803.

450 Chen S, Dong H, Fan Y, Li W, Cohen Y, 2006. Dry mycelium of *Penicillium chrysogenum*
 451 induces expression of pathogenesis-related protein genes and resistance against wilt
 452 diseases in Bt transgenic cotton. *Biological Control* **39**, 460-464.

453 Di Pietro A, Madrid MP, Caracuel Z, Delgado-Jarana J, Roncero MIG, 2003. *Fusarium*
 454 *oxysporum*: exploring the molecular arsenal of a vascular wilt fungus. *Molecular Plant*
 455 *Pathology* **4**, 315-325.

456 Domsch KH, Gams W, Anderson TH, 1980. *Compendium of Soil Fungi*. Academic Press,
 457 London, UK.

458 Dubey SC, Suresh M, Singh B, 2007. Evaluation of *Trichoderma* species against *Fusarium*
 459 *oxysporum* f. sp *ciceris* for integrated management of chickpea wilt. *Biological Control* **40**,
 460 118-127.

461 Fravel D, Olivain C, Alabouvette C, 2003. *Fusarium oxysporum* and its biocontrol. *New*
 462 *Phytologist* **157**, 493-502.

463 Fuchs JG, Moenne-Loccoz Y, Defago G, 1999. Ability of nonpathogenic *Fusarium*
 464 *oxysporum* Fo47 to protect tomato against *Fusarium* wilt. *Biological Control* **14**, 105-110.

465 Gaiero JR, McCall CA, Thompson KA, Day NJ, Best AS, Dunfield KE, 2013. Inside the
 466 root microbiome: bacterial root endohytes and plant growth promotion. *American Journal*
 467 *of Botany* **100**, 1738-1750.

468 Geiser DM, Frisvad JC, Taylor JW, 1998. Evolutionary Relationships in *Aspergillus*
 469 Section Fumigati Inferred from Partial β -Tubulin and Hydrophobin DNA Sequences.
 470 *Mycologia* **90**, 831-845.

471 Glass NL, Donaldson GC, 1995. Development of primer sets designed for use with the
 472 PCR to amplify conserved genes from filamentous ascomycetes. *Applied and*
 473 *Environmental Microbiology* **61**, 1323-1330.

474 Gouba N, Raoult D, Drancourt M, 2013. Plant and Fungal Diversity in Gut Microbiota as
 475 Revealed by Molecular and Culture Investigations. *PloS one* **8**.

476 Gouba N, Raoult D, Drancourt M, 2014. Eukaryote Culturomics of the Gut Reveals New
 477 Species. *PloS one* **9**.

478 Hardoim PR, Andreote FD, Reinhold-Hurek B, Sessitsch A, van Overbeek LS, van Elsas
 479 JD, 2011. Rice root-associated bacteria: insights into community structures across 10
 480 cultivars. *Fems Microbiology Ecology* **77**, 154-164.

481 Huang B, Harper DAT, Hammer O, 2013. Introduction to PAST, a comprehensive statistics
 482 software package for paleontological data analysis. *Acta Palaeontologica Sinica* **52**, 161-
 483 181.

484 Huang CC, Lindhout P, 1997. Screening for resistance in wild *Lycopersicon* species to
 485 *Fusarium oxysporum* f sp *lycopersici* race 1 and race 2. *Euphytica* **93**, 145-153.

486 Inceoglu O, Salles JF, van Elsas JD, 2012. Soil and Cultivar Type Shape the Bacterial
 487 Community in the Potato Rhizosphere. *Microbial Ecology* **63**, 460-470.

488 Kiffer E, Morelet M, 1997. *Les Deuteromycetes: Classification et clefs d' Identification*
 489 *Generique*. INRA Editions, Paris, France.

490 Kinkel LL, Bakker MG, Schlatter DC, 2011. A Coevolutionary Framework for Managing
 491 Disease-Suppressive Soils, in: VanAlfen NK, Bruening G, Leach JE (eds), *Annual Review*
 492 *of Phytopathology*, Vol 49, pp. 47-67.
 493 Lakshmanan V, Selvaraj G, Bais HP, 2014. Functional Soil Microbiome: Belowground
 494 Solutions to an Aboveground Problem. *Plant Physiology* **166**, 689-700.
 495 Larena I, Melgarejo P, De Cal A, 2003. Drying of conidia of *Penicillium oxalicum*, a
 496 biological control agent against *Fusarium* wilt of tomato. *Journal of Phytopathology* **151**,
 497 600-606.
 498 Lebeis SL, Paredes SH, Lundberg DS, Breakfield N, Gehring J, McDonald M, Malfatti S,
 499 del Rio TG, Jones CD, Tringe SG, Dangl JL, 2015. Salicylic acid modulates colonization
 500 of the root microbiome by specific bacterial taxa. *Science* **349**, 860-864.
 501 Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, Tremblay J,
 502 Engelbrektson A, Kunin V, del Rio TG, Edgar RC, Eickhorst T, Ley RE, Hugenholtz P,
 503 Tringe SG, Dangl JL, 2012. Defining the core *Arabidopsis thaliana* root microbiome.
 504 *Nature* **488**, 86-90.
 505 Mazzola M, 2002. Mechanisms of natural soil suppressiveness to soilborne diseases.
 506 *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology*
 507 **81**, 557-564.
 508 Mazzola M, 2004. Assessment and management of soil microbial community structure for
 509 disease suppression. *Annual Review of Phytopathology* **42**, 35-59.
 510 Mendes R, Kruijt M, de Bruijn I, Dekkers E, van der Voort M, Schneider JHM, Piceno
 511 YM, DeSantis TZ, Andersen GL, Bakker PAHM, Raaijmakers JM, 2011. Deciphering the
 512 Rhizosphere Microbiome for Disease-Suppressive Bacteria. *Science* **332**, 1097-1100.
 513 Michielse CB, Rep M, 2009. Pathogen profile update: *Fusarium oxysporum*. *Molecular*
 514 *Plant Pathology* **10**, 311-324.

515 Nallanchakravarthula S, Mahmood S, Alstrom S, Finlay RD, 2014. Influence of Soil Type,
 516 Cultivar and *Verticillium dahliae* on the Structure of the Root and Rhizosphere Soil Fungal
 517 Microbiome of Strawberry. *PloS one* **9**, e111455-e111455.
 518 Nam Y-J, Kim H, Lee J-H, Yoon H, Kim J-G, 2015. Metagenomic analysis of soil fungal
 519 communities on Ulleungdo and Dokdo Islands. *The Journal of General and Applied*
 520 *Microbiology* **61**, 67-74.
 521 Nel B, Steinberg C, Labuschagne N, Viljoen A, 2006a. Isolation and characterization of
 522 nonpathogenic *Fusarium oxysporum* isolates from the rhizosphere of healthy banana plants.
 523 *Plant Pathology* **55**, 207-216.
 524 Nel B, Steinberg C, Labuschagne N, Viljoen A, 2006b. The potential of nonpathogenic
 525 *Fusarium oxysporum* and other biological control organisms for suppressing fusarium wilt
 526 of banana. *Plant Pathology* **55**, 217-223.
 527 Nicoletti R, Lopez-Gresa MP, Manzo E, Carella A, Ciavatta ML, 2007. Production and
 528 fungitoxic activity of Sch 642305, a secondary metabolite of *Penicillium canescens*.
 529 *Mycopathologia* **163**, 295-301.
 530 Oksanen J, Blanchet FG, Kindt GR, Legendre P, Minchin P, O'Hara RB, Simpson G,
 531 Solymos P, Stevens H, Wagner H, 2013. Vegan: Community Ecology Package.
 532 Ozminkowski R, 2004. Pedigree of variety Heinz 1706. Report of the Tomato Genetics
 533 Cooperative. Coop., p. 26.
 534 Paulitz TC, Belanger RR, 2001. Biological control in greenhouse systems. *Annual Review*
 535 *of Phytopathology* **39**, 103-133.
 536 Penton CR, Gupta VVSR, Tiedje JM, Neate SM, Ophel-Keller K, Gillings M, Harvey P,
 537 Pham A, Roget DK, 2014. Fungal Community Structure in Disease Suppressive Soils
 538 Assessed by 28S LSU Gene Sequencing. *PloS one* **9**.

539 Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH, 2013. Going back to the
 540 roots: the microbial ecology of the rhizosphere. *Nature Reviews Microbiology* **11**, 789-799.
 541 Punja ZK, Utkhede RS, 2003. Using fungi and yeasts to manage vegetable crop diseases.
 542 *Trends in Biotechnology* **21**, 400-407.
 543 Radhakrishnan R, Shim K-B, Lee B-W, Hwang C-D, Pae S-B, Park C-H, Kim S-U, Lee
 544 C-K, Baek I-Y, 2013. IAA-Producing *Penicillium* sp NICS01 Triggers Plant Growth and
 545 Suppresses *Fusarium* sp.-Induced Oxidative Stress in Sesame (*Sesamum indicum* L.).
 546 *Journal of Microbiology and Biotechnology* **23**, 856-863.
 547 Rivera MC, Lopez MV, Lopez SE, 2009. Mycobiota from *Cyclamen persicum* and its
 548 interaction with *Botrytis cinerea*. *Mycologia* **101**, 173-181.
 549 Sabuquillo P, De Cal A, Melgarejo P, 2006. Biocontrol of tomato wilt by *Penicillium*
 550 oxalicum formulations in different crop conditions. *Biological Control* **37**, 256-265.
 551 Salvioli A, Zouari I, Chalot M, Bonfante P, 2012. The arbuscular mycorrhizal status has
 552 an impact on the transcriptome profile and amino acid composition of tomato fruit. *Bmc*
 553 *Plant Biology* **12**.
 554 Samson RA, Seifert KA, Kuijpers AFA, Houbraken J, Frisvad JC, 2004. Phylogenetic
 555 analysis of *Penicillium* subgenus *Penicillium* using partial P-tubulin sequences. *Studies in*
 556 *Mycology*, 175-200.
 557 Spadaro D, Gullino ML, 2005. Improving the efficacy of biocontrol agents against
 558 soilborne pathogens. *Crop Protection* **24**, 601-613.
 559 Spence C, Alff E, Johnson C, Ramos C, Donofrio N, Sundaresan V, Bais H, 2014. Natural
 560 rice rhizospheric microbes suppress rice blast infections. *BMC Plant Biology* **14**, 130-130.
 561 Srinivasan K, Gilardi G, Garibaldi A, Gullino ML, 2009. Bacterial antagonists from used
 562 rockwool soilless substrates suppress *Fusarium* wilt of tomato. *Journal of Plant Pathology*
 563 **91**, 147-154.

564 Turner TR, James EK, Poole PS, 2013. The plant microbiome. *Genome Biology* **14**, 1-11.
 565 von Arx JA, 1981. *The Genera of Fungi Sporulating in Pure Culture*. Cramer, Vaduz,
 566 Germany.
 567 Vos CM, Yang Y, De Coninck B, Cammue BPA, 2014. Fungal (-like) biocontrol organisms
 568 in tomato disease control. *Biological Control* **74**, 65-81.
 569 Vujanovic V, Hamelin RC, Bernier L, Vujanovic G, St-Arnaud M, 2007. Fungal diversity,
 570 dominance, and community structure in the rhizosphere of clonal *Picea mariana* plants
 571 throughout nursery production chronosequences. *Microbial Ecology* **54**, 672-684.
 572 White TJ, Bruns T, Lee S, Taylor J, 1990. Amplification and direct sequencing of fungal
 573 ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and*
 574 *Applications* **18**, 315-322.
 575 Yao H, Wu F, 2010. Soil microbial community structure in cucumber rhizosphere of
 576 different resistance cultivars to fusarium wilt. *FEMS Microbial Ecology* **72**, 456-463.

TABLES

Table 1. Physical and chemical characteristics of the soils considered in this study.

	Soil A	Soil B
Origin	Albenga (SV), Liguria, northern Italy	Rosta (TO), Piedmont, northern Italy
Geographical coordinates	44.067171 N, 8.212949 E	45.074190 N, 7.461910 E
pH	7.22	7.60
sand:silt:clay (%)	60.0:10.7:29.3	60.0:16.7:23.3
C organic (%)	3.18	0.60
N total(‰)	1.68	1.54
Ca (mg/Kg)	3903.80	4036.90
Mg (mg/Kg)	726.00	469.40
K (mg/Kg)	834.20	116.00
Na(mg/Kg)	895.20	149.40
P (mg/Kg)	16.90	10.20
B (mg/Kg)	0.70	1.00
Fe (mg/Kg)	93.70	19.60
Conductivity (mS/cm)	9.90	0.46

Table 2. NPMANOVA analysis of Bray-Curtis dissimilarities in the rhizospheric (A) and in the endophytic (B) fungal community structure in relation to soil type, genotype and their interaction ($p < 0.05$). SSquares = sum of squares; df = degrees of freedom; MSquares = mean sum of squares; F = value by permutation; p = p value based on 9999 permutations.

A.

Permutation N: 9999	SSquares	df	MSquares	F	p
Genotype	0.20288	1	0.20288	2.27	0.0798
Soil	0.4797	1	0.4797	5.3671	0.0005
Interaction	0.40047	1	0.40047	4.4807	0.0033
Residual	0.71502	8	0.089377		
Total	1.7981	11			

B.

Permutation N: 9999	SSquares	df	MSquares	F	p
Soil	0.7946	1	0.7946	3.3601	0.0013
Genotype	1.1468	1	1.1468	4.8496	0.0001
Interaction	0.7449	1	0.7449	3.1501	0.0022
Residual	1.8919	8	0.2364		
Total	4.5783	11			

Table 3. *t*-test evaluating the effect of the soil type, genotype, and pathogen addition (+*Fol*) on the rhizospheric (A) and endophytic (B) fungal diversity (Shannon index). *t* = *t* score; df = degrees of freedom; *p* = *p* value.

A.

Genotype	<i>t</i>	<i>p</i>		38
SA vs. RA	-0.16	0.8	ns	39
SB vs. RB	0.73	0.5	ns	
Soil				40
SA vs. SB	2.99	0.04	*	41
RA vs. RB	3.51	0.02	*	
+ <i>Fol</i>	<i>t</i>	<i>p</i>		42
RAF vs. RA	0.98	0.3	ns	43
RBF vs. RB	-0.78	0.4	ns	
				44

B.

Genotype	<i>t</i>	<i>p</i>		48
SA vs. RA	-0.083	0.93	ns	49
SB vs. RB	3.71	0.02	*	
Soil				50
SA vs. SB	-7.0	0.002	**	51
RA vs. RB	-0.80	0.47	ns	
+ <i>Fol</i>	<i>t</i>	<i>p</i>		52
RAF vs. RA	-0.11	0.91	ns	53
RBF vs. RB	-0.94	0.39	ns	
				54

** *P* < 0.01; * *P* < 0.05; ns non significant

CAPTION TO ILLUSTRATIONS

Figure 1. Fungal total load (CFU per gram of soil dry weight) detected in the rhizosphere (A) and in the roots (B) of tomato plants cultivated on the two soils. SA (Susceptible cultivar/ soil A), SB (Susceptible cultivar/soil B), RA (Resistant cultivar/soil A), RB (Resistant cultivar /soil B). Results are expressed as mean $\pm$ s.e. and analyzed through one-way analysis of variance (ANOVA), Bonferroni post hoc ($p < 0.05$).

Figure 2. Diversity (relative abundance in percentage) in the rhizosphere (A) and in the roots (B) of species contributing to the differences observed in all treatments. SA (Susceptible cultivar/ soil A), SB (Susceptible cultivar/soil B), RA (Resistant cultivar/soil A), RB (Resistant cultivar /soil B). (Resistant cv/ Conducive soil), RAF (Resistant cv/soil A/*Fol*), RBF (Resistant cv/soil B/*Fol*).

Figure 3. DNA fingerprinting profiles generated from genomic DNA of 20 *Fusarium oxysporum* isolates with the microsatellite primer M13. Twenty μ L of PCR products were separated by electrophoresis on a 1.5% agarose gel for 3 h at 45 V/cm². M = 1kb DNA ladder; 1 = *Fusarium oxysporum* f. sp. *lycopersici* used for the inoculum; 2 – 20 *Fusarium oxysporum* isolates from both rhizospheric and root samples. Arrows indicate the pathogen retrieved in the samples.

Figure 4. Effect of the addition of *Fol* on the fungal total load (CFU per gram of soil dry weight) in the rhizosphere and in the roots for the treatment indicated. RA (Resistant/soil A), RAF (Resistant/soil A/*Fol*), RB (Resistant/soil B), RBF (Resistant/soil B/*Fol*). Results are expressed as mean $\pm$ s.e. and analyzed through one-way analysis of variance (ANOVA), Bonferroni post hoc ($p < 0.05$).

Figure 5. Effect of the addition of *Fol* on the load (CFU per gram of soil dry weight) of single genera/groups in the rhizosphere and in the roots for the treatment indicated. Results are expressed

as mean $\pm$ s.e. and analyzed through unpaired *t*-test RA (Resistant/soil A) vs RAF (Resistant/soil A/*Fol*) (capital letters) and RB (Resistant/soil B) vs RBF (Resistant/soil B/*Fol*) (lower case letters).

Figure 1. Number of total CFU per gram of dry soil detected in the rhizosphere (A) and in the roots (B) of tomato plants cultivated on the two soils. SA (Susceptible cultivar/ soil A), SB (Susceptible cultivar/soil B), RA (Resistant cultivar/soil A), RB (Resistant cultivar /soil B). Results are expressed as mean $\pm$ s.e. and analyzed through one-way analysis of variance (ANOVA), Bonferroni post hoc ($p < 0.05$).

A.

B.

Figure 2. Diversity (in percentage of abundance) in the rhizosphere (A) and in the roots (B) of species contributing to the differences observed in all treatments. SA (Susceptible cultivar/ soil A), SB (Susceptible cultivar/soil B), RA (Resistant cultivar/soil A), RB (Resistant cultivar /soil B). (Resistant cv/ Conductive soil), RAF (Resistant cv/soil A/*Fol*), RBF (Resistant cv/soil B/*Fol*).

26 B.

Figure 3. DNA fingerprinting profiles generated from genomic DNA of 20 *Fusarium oxysporum* isolates with the microsatellite primer M13. Twenty µL of PCR products were separated by electrophoresis on a 1.5% agarose gel for 3 h at 45 V/cm². M = 1kb DNA ladder; 1 = *Fusarium oxysporum* f. sp. *lycopersici* used for the inoculum; 2 – 20 *Fusarium oxysporum* isolates from both rizospheric and root samples. Arrows indicate the pathogen retrieved in the samples.

Figure 4. Effect of the addition of *Fol* on the total fungal load of the rhizosphere and in the roots for the treatment indicated. RA (Resistant/soil A), RAF (Resistant/soil A/*Fol*), RB (Resistant/soil B), RBF (Resistant/soil B/*Fol*). Results are expressed as mean $\pm$ s.e. and analyzed through one-way analysis of variance (ANOVA), Bonferroni post hoc ($p < 0.05$).

A.

B.

72 **Figure 5.** Effect of the addition of *Fol* on single genera/groups in the rhizosphere and in
73 the roots for the treatment indicated. Results are expressed as mean $\pm$ s.e. and analyzed
74 through unpaired *t-test* RA (Resistant/soil A) vs RAF (Resistant/soil A/*Fol*) (capital
75 letters) and RB (Resistant/soil B) vs RBF (Resistant/soil B/*Fol*) (lower case letters).

Rhizosphere

Roots

76

77

78

79

80