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Rhizospheric changes of fungal and bacterial communities in relation to soil health of multi-generation apple orchards

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HIGHLIGHTS

- Microbial changes linked to plant health were analyzed in apple tree rhizosphere.
- Microbial communities of native and re-colonized soils were compared.
- Both bacterial and fungal communities resulted modified in re-colonized soils.
- *Pseudomonas* and *Novosphingobium* were mainly involved in bacterial changes.
- Changes in composition within *Fusarium* were linked to plant health.

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ABSTRACT

The study focused on changes of rhizosphere microbial communities in apple trees in long-term replanted orchards of Central Europe, aiming at developing cropping practices to mitigate replant problems. It started from the evidence of a previous study which showed that a slight modification of root-colonizing fungal communities was responsible for a great increase of plant growth in soil samples which had previously been subjected to a gamma-irradiation cycle (25 kGy for 8 h), as compared to that observed in the corresponding untreated native soils.

The study was performed on rhizospheric soil from nine multi-generation apple orchards after a plant growth assay with M9 rootstock plantlets. PCR-DGGE analysis of soil DNA was performed to evaluate fungal and bacterial communities in fallow and replanted soils, as well as corresponding gamma-irradiated samples. Findings showed that rhizospheric fungal and bacterial communities within apple orchards did not differ according to their position within the orchard; while, they showed a shift in the gamma-irradiated soils. *Pseudomonas fluorescens*, *Pseudomonas tolasii*, *Pseudomonas* spp. and *Novosphingobium* spp. were the bacteria which were mainly attributed to this change. A shifting in composition of *Fusarium* communities toward *Fusarium oxysporum* and *Fusarium equiseti* resulted the most linked to the changes at rhizosphere level after re-colonization; to the contrary, *Fusarium venenatum* and *Fusarium avenaceum*, *Truncatella* sp. and *Gibellolopsis* sp., only occurred in native soils. Findings of this study suggest that disturbance events such as a gamma-irradiation can modify microbial communities in long-term apple orchards thus allowing a soil re-colonization suitable to increase soil suppressiveness.

1. Introduction

Progressive reduction of yield and quality is a worldwide problem for fruit tree cropping systems in intensively grown areas. It is defined as replant disease, soil sickness or, more generically, replant problems or disorders of fruit tree orchards. It represents a multifactorial phenomenon about which studies conducted in open field with soil fumigants (Braun et al., 2010; Browne et al., 2006; Mai et al., 1976) and pasteurized soil in bioassay tests (Mazzola, 1998; Utkhede and Li, 1988) have provided extensive evidence that biotic components play a primary role. However, the complexity of beneficial and pathogenic microbial interaction with plants, mediated by abiotic and nutritional factors affecting the physiological state of crops, still makes it difficult to identify an effective control strategy for this problem, which is increasing progressively worldwide.

The scope of this study falls within a research framework aimed at developing agronomic tools which can increase the beneficial

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microbial components already present in soils, and induce some plant–microorganism interaction able to enhance soil health in long-term fruit-tree orchards.

Although evidence of replant problems in fruit tree orchards had been reported for a long time in Europe (Gilles and Bal, 1988; Hoestra, 1968; Savory, 1966), there was little interest in the losses caused by this multifactorial problem until early 2000 (Forge et al., 2003; Manici et al., 2003). This interest followed a period marked by significant reduction in land invested with fruit tree, which occurred during the 90s in EU-15 (the European Union of 15 Member States, before 1 May 2004; Vidal and Ribaille, 2001). In fact, the intensive apple-growing areas of Central Europe, characterized by temperate climate and soil management, which guarantee soil organic matter content have, for a long time, been able to slow down apple replant disease. In addition to this, the EU Common Agricultural Policy over the last five to ten years has aimed at counteracting the risks of environmental degradation and promoting sustainability of agro-ecosystems (European Commission, 2012). This trend is consistent with the progressive conversion to organic management of fruit tree orchard since 2000 and is further confirmed by the annual increase in sales of organic fruit and vegetables in Europe (Organic Monitor, 2008).

At the current time in specialized apple-growing regions of Europe, great efforts are made to respect the environment and to identify innovative cropping practices for controlling apple replant problems (Kelderer et al., 2012). One of the most innovative research approaches is based on manipulation of microbial communities already present in soils. It should be possible to achieve this by increasing the reducing negative biotic components and increasing beneficial populations in soil using agronomic tools such as organic amendments and cover crops. This, on the one hand, implies the application of biotechnologies to the investigation of soil microbial communities and their response to cropping practices; and on the other hand, imposes the use of natural resources as an environmentally friendly means to affect soil biota.

A previous survey in long-term replanted orchards of Central Europe showed that a slight modification of root endophytic fungal communities was responsible for a great increase in plant growth observed in soil samples which had been subjected to a gamma-irradiation cycle, compared to that in corresponding native soils (Manici et al., 2013). In relation to the fungi isolated from apple roots in that previous survey, although *Cylindrocarpon*-like fungi and *Pythium* spp. were negatively correlated to plant growth, more than 60% of root inhabiting fungi were not. Finally, gamma-irradiated soil samples, used as health control in that survey, induced much higher rooting (Fig. 1) and plant growth (about 35%) than that observed in the two corresponding native soils (fallow and replant soil). As in the previous study plant growth increase in the gamma-irradiated treatment could not be explained by the reduction of soil-borne pathogens alone (Manici et al., 2013) and based on the concept that plant growth with bioassay is one of the main indicators soil suppressiveness (Nielsen and Winding, 2002), this second part was focused on rhizosphere microbial communities aiming at analyzing their changes after soil re-colonization. The medium term objectives of the current study were: (i) to evaluate whether modification of bacterial and fungal communities of rhizosphere occurred in gamma-irradiation treatments as compared to native soil; (ii) to identify which bacterial and fungal populations were more involved in that shift; (iii) to investigate changes of *Fusarium* spp. at rhizosphere level, since they were the most abundant root inhabiting fungi in the previous study.

2. Materials and methods

2.1. Soil sampling

The study was performed on rhizosphere soil samples collected at the end of a bioassay test, carried out in pots with rooted cuttings of clonal M9 rootstock at the Laimburg Research Centre in South Tyrol (Italy). Soil samples used for this bioassay came from nine multi-generation apple orchards located in three main apple growing areas of central Europe: Rhineland Palatinate (Germany), Styria (Austria), and South Tyrol (Italy). The complete list of physical, chemical and biochemical features of soil samples was reported in the first part of this study published in 2013 (Manici et al.). Soil texture varied from sandy-loam to clay-loam and soil organic matter (SOM) content was generally high ranging from 3% to 9% (Manici et al., 2013). Each orchard was split into three soil treatments, which were:

- replanted soil (R), taken from the 0 to 30 cm top layer under the apple tree within the orchards;
- fallow soil (F), taken from the 0 to 30 cm top layer in strip row or other fallow plots within each orchard or on the edges (plots with natural vegetal cover which did not host apple tree since thirty years);
- gamma-irradiated soil (S), consisting in replanted soil subjected to one sanitization gamma-ray cycle (25 kGy) for 8 h, inside an irradiation cell as described in Manici et al. (2013). This

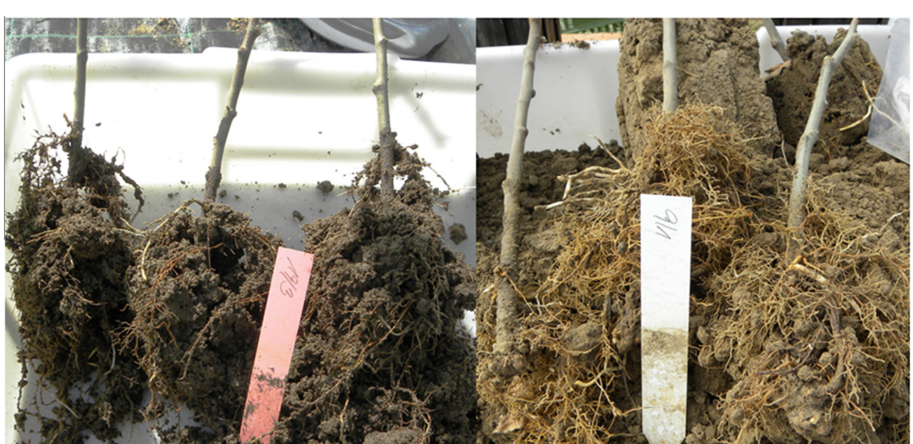

Fig. 1. Root development of M9 rootstock plantlets grown on replanted soil (left, pink label) as compared to that obtained on gamma-irradiated soil (right, white label). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)