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Short communication

Plant pathogens but not antagonists change in soil fungal communities across a land abandonment gradient in a Mediterranean landscape

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ABSTRACT

We assessed whether the presence and abundance of plant pathogens and antagonists change in soil fungal communities along a land abandonment gradient. The study was carried out in the Cilento area (Southern Italy) at a site with three different habitats found along a land abandonment gradient: agricultural land, Mediterranean shrubland and woodland. For all microbiological substrates the colony forming units were about 3.1×10^6 g^{-1} soil for agricultural land and about 1.1×10^6 g^{-1} soil for Mediterranean shrubland and woodland. We found the following genera in all habitats: *Cladosporium, Mortierella, Penicillium* and *Trichoderma*. In agricultural land, the significantly most abundant fungus genera were *Aspergillus, Fusarium, Cylindrocarpon* and *Nectria*; in Mediterranean shrubland, *Rhizopus* and *Trichoderma*; and in woodland, *Bionectria, Mortierella, Cladosporium, Diplodia, Paecilomyces, Penicillium* and *Trichoderma*. We found a total of 8, 8 and 9 species of fungal antagonist, and 16, 6 and 6 species of fungal plant pathogens in agricultural land, Mediterranean shrubland and woodland respectively. Fungal plant pathogens decreased significantly over a land abandonment gradient, while we no found significant differences among fungal antagonists in the three habitats. We conclude that a decrease in the number of fungal pathogen species occurs when formerly cultivated areas are abandoned. On the other hand, fungal antagonists seem not to be affected by this process.
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1. Introduction

Land abandonment is a common process observed in many Mediterranean landscapes and is one of the dominant factors affecting the biodiversity and functioning of soil ecosystems (Zornoza et al., 2009; Lopez-Sangil et al., 2011). This process has important consequences for soil ecosystem dynamics, influencing functions and services such as nutrient cycling, carbon sequestration, decomposition of recalcitrant organic matter, transport of nutrients, microbial diversity, interaction and abundance of pathogenic and antagonistic microorganisms (Raaijmakers et al., 2009).

The Mediterranean basin is characterized by different climatic regions and plant communities. In this area a large number of plant pathogens may cause numerous plant diseases, which can be widespread or limited in their distribution, and result in more or less severe impact on yield or quality of crop production (Pautasso et al., 2015). In the Mediterranean basin recent plant health emergencies include the outbreaks of the oomycete *Phytophthora ramorum* (Brasier and Webber, 2010), the fungus *Hymenoscyphus fraxineus* (Gross et al., 2014) and the bacterium *Xylella fastidiosa* (Bosso et al., 2016a,b). The growth and activity of plant pathogens in soil can be inhibited by several antagonistic microorganisms such as bacteria belonging to genera *Pseudomonas*, *Burkholderia* and *Bacillus,* and fungi belonging to genera *Trichoderma*, *Gliocladium*, *Aspergillus*, *Cladosporium*, *Paecylomices* and *Penicillium* (Raaijmakers et al., 2009). These microorganisms, also called biocontrol agents, may affect the population density and metabolic activities of several plant pathogens.

Fungi can play an important role in soil ecosystem processes and influence vegetation dynamics by acting as pathogens or as mutualistic symbionts (van der Wal et al., 2006). The abundance and diversity of plant pathogens and antagonists in soil fungal communities are related to the quantity and quality of the rhizodeposits and to the outcome of the microbial interactions that occur in the rhizosphere, i.e. the narrow region of soil that is directly

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influenced by root secretions and associated soil microorganisms (Raaijmakers et al., 2009).

Although numerous studies have shown that changes in land use have a significant effect on microbial communities (van der Wal et al., 2006; Rey Benayas et al., 2007; Jangid et al., 2011), how land abandonment may affect the abundance of specific taxonomic groups of plant pathogens and antagonists in soil fungal communities has so far attracted little attention.

The aim of this study was to assess whether the presence and abundance of plant pathogens and antagonists change in soil fungal communities along a land abandonment gradient.

2. Materials and methods

2.1. Study area

The study was carried out in the buffer zone of the Cilento Vallo di Diano and Alburni National Park, a ca. 200-ha flat internal area at ca. 500 m a.s.l. (UTM coordinates: 33 T–507520.47 m E; 4473925.08 m N). The area was characterized by a Mediterranean climate with dry summers, and rainy autumns and winters (Romano et al., 2010). Three habitats corresponding to a land abandonment gradient were identified: agricultural land, Mediterranean shrubland and woodland.

Typically, the abandonment of agricultural or pastoral systems in the Mediterranean leads to landscape changes through secondary vegetation succession comprising scrub encroachment (i.e. a shrubland transitional stage) and forest re-growth (Moreira and Russo, 2007). In our study sites such stages were represented by the above-mentioned Mediterranean shrubland and woodland.

Agricultural land was mainly characterized by *Sulla coronaria* (L.) Medik.; Mediterranean shrubland by *Fraxinus ornus* L. *Myrtus communis* L. *Phillyrea latifolia* L. *Pistacia lentiscus* L. and *Spartium junceum* L.; and woodland by *Quercus pubescens* Willd. and *Fraxinus ornus* L. Agricultural land was not subjected to crop rotation, intercropping, tillage management practices or a combination of those regimes.

2.2. Experimental design and physico-chemical soil properties

Nine 5 × 10 m plots – 3 for each habitat – were used for the experiments. In each experimental plot, we randomly selected three 1 m^2 square plots ca. 3 m apart from one another. From each subplot, representing our replicates, we took 9 soil samples (7 cm diameter, 0–5 cm depth and sieved at 2 mm mesh size) (e.g. Maggi et al., 2005; Rutigliano et al., 2013). Physical and chemical soil properties were measured from air-dried soil samples according to standard techniques (Sparks, 1996). The texture was evaluated according to USDA Textural Soil Classification (Soil Survey Staff, 1975). Electrical conductivity (EC) and pH were measured in 1:5 and 1:2.5 soil-water suspensions, respectively. The nitrate (NO_3^--N) was extracted at pH 1.0–1.5 with a mixture of potassium sulphate and sulphuric acid, and the ammonia was determined by distillation with magnesium oxide at 25 °C (Bremner and Shaw, 1955). Total Organic Carbon (TOC) content was assayed by the chromic acid titration method while total N (TN) was determined by the ash combustion procedure with a Fisons 1108 Elemental Analyzer (Alef and Nannipieri, 1995). C:N was calculated as ratio between TOC and TN. Further details about physico-chemical soil analysis are available in Bosso et al. (2015a).

2.3. Isolation of fungi from soils

To evaluate presence and abundance of plant pathogens and antagonists in soil fungal communities, soil samples were treated with the soil dilution plate method (dry soil/water ratio 1:1000, Maggi et al., 2005). This method is simple and rapid, gives reasonably repeatable results and yields excellent comparative data (e.g. Maggi et al., 2005; Rutigliano et al., 2013). For each soil sample, five replicate plates were prepared with 0.1 mL/plate for each of the following substrates: 20 mL potato dextrose agar (PDA, BD); Rose Bengal Agar Base (RBA, Difco); malt extract agar (MEA, Difco); and Oatmeal Agar (OA, Difco). These are the principal microbiological substrates used for fungal strains isolation for soils (Alef and Nannipieri, 1995; Paul, 2006). The soil dilution plate method (dry soil/water ratio 1:890) was also used to isolate fungal strains by heating the suspension at 55 °C for 30 min and five plates were prepared with 0.1 mL/pl and 20 mL of PDA, RBA, MEA and OA. To avoid bacterial contamination, all media were supplemented with ampicillin (100 γ/mL substrate). Plates were incubated at 25 °C, and the colony forming units (CFU) were counted after 5 days of incubation at 25 °C and isolated in pure culture for species identification through molecular and morphological analyses (Maggi et al., 2005). All fungal strains isolated by soils were stored on MEA and PDA in slant tubes at 20 °C (e.g.: Bosso et al., 2015b; Bosso et al., 2016c) at the laboratories of Forest Pathology of the Department of Agriculture (University of Naples Federico II, Italy). Plate culture-based methods for isolation and cultivation of fungal strains in microbiological substrates are desirable when live cultures are needed for subsequent studies such as e.g. molecular identification. The major limitation of plate culture-based methods is that some fungal species may not be cultivated under the growth conditions used (Boundy-Mills, 2013).

2.4. Molecular identification of the fungal strains isolated from soils

The fungal strains developed on 10^{-4} dilution were identified by molecular and morphological analyses (e.g.: Gomez et al., 2007). Molecular identification was carried out by transferring each fungus from pure culture to PDA plates and incubating it at 25 °C for 7 days. Two plugs were taken from fungal plates, placed in flasks with potato dextrose broth (PDB, Biosigma), and incubated for 30 days at 25 °C on orbital shaker at 120 rpm. Mycelium harvested from flasks was freeze-dried and stored in falcon tubes. DNA extraction was done following de Graaff et al. (1988). Genetic analysis of ribosomal DNA was determined by PCR of the internal transcribed spacer (ITS) sequences employing the most common primers used to detect fungal strains: ITS1F (5′-TCCGTAGGTGAACCTGCGG-3′), ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′), ITS3 (5′-GCATCGATGAA-GAACGCAGC-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). These primers successfully amplified all higher fungal species tested (Manter and Vivanco, 2007; Borman et al., 2008; Bosso et al., 2011; Ihrmark et al., 2012; Hechmi et al., 2016). The following parameters were used in thermocycler amplification: 1 min initial denaturation at 94 °C, followed by 30 cycles of 1 min denaturation at 94 °C, 1 min primer annealing at 50 °C, 90 s for extension at 72 °C and final extension period of 7 min at 72 °C. The polymerase chain reaction (PCR) product was analyzed for agarose gel electrophoresis and quantified by Qubit® 2.0 Fluorometer. The PCR product was sent to Eurofins MWG Operon for sequencing. Finally, DNA sequences were aligned using the Basic Local Alignment Search Tool (BLAST) which recognises regions of local similarity between sequences (http://blast.ncbi.nlm.nih.gov/Blast.cgi). After molecular identification the fungal cultures were also characterized according to their macroscopic and microscopic features (e.g.: Romano et al., 2016; Hechmi et al., 2016).

We calculated the relative abundance of genera of plant pathogens and antagonists in soil fungal communities along a land abandonment gradient (Elmholt, 1996). This analysis was carried out for each experimental plot in the three different habitats.

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