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Indirect host effect on ectomycorrhizal fungi: Leaf fall and litter quality explain changes in fungal communities on the roots of co-occurring Mediterranean oaks

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ABSTRACT

Host trees can modify their soil abiotic conditions through their leaf fall quality which in turn may influence the ectomycorrhizal (ECM) fungal community composition. We investigated this indirect interaction using a causal modelling approach. We identified ECM fungi on the roots of two coexisting oak species growing in two forests in southern Spain – *Quercus suber* (evergreen) and *Quercus canariensis* (winter deciduous)-using a PCR-based molecular method. We also analysed the leaf fall, litter and soil sampled beneath the tree canopies to determine the concentrations of key nutrients. The total mycorrhizal pool was comprised of 69 operational taxonomic units (OTUs). *Tomentella* and *Russula* were the most species-rich, frequent and abundant genera. ECM fungi with epigeous and resupinate fruiting bodies were found in 60% and 34% of the identified mycorrhizas, respectively. The calcium content of litter, which was significantly higher beneath the winter-deciduous oak species due to differences in leaf fall quality, was the most important variable for explaining ECM species distribution. The evaluation of alternative causal models by the d-sep method revealed that only those considering indirect leaf fall-mediated host effects statistically matched the observed covariation patterns between host, environment (litter, topsoil, subsoil) and fungal community variables.

1. Introduction

Mycorrhizal symbioses are essential for oak trees for acquiring nutrients under natural conditions (Smith and Read, 1997). Ectomycorrhizal (ECM) fungi supply plants with water and nutrients by increasing their foraging area and absorbing efficiency, and they provide an ample range of other beneficial effects as well, in exchange for photosynthesised products (Schutzendubel and Polle, 2002; Frey-Klett et al., 2005; Egerton-Warburton et al., 2007; Finlay, 2008). ECM communities contain a high diversity of fungal taxa (Taylor and Alexander, 2005), which are associated with a variety of functional strategies that contribute to forest ecosystem stability and functioning (Perry et al., 1989; Nara, 2006).

During the last decade, numerous studies have tried to unveil the role that natural factors, such as environmental conditions and host plant community composition, have on the assemblage of ECM communities (Conn and Dighton, 2000; Dickie and Reich, 2005; Buée et al., 2007). Soil abiotic conditions, namely, soil moisture, pH and nutrient availability, influence the performance and assemblage of fungal species (Brearley, 2006; Cavender-Bares et al., 2009). Host trees may directly affect the assemblage of their mycorrhizal community by exerting a selection for mycorrhizal species (Ishida et al., 2007; Tedersoo et al., 2008).

In a recent study Morris et al. (2008) studied the separate effects of soil conditions and host tree species on the composition of ECM communities in a California mixed-oak forest and concluded that both explained a significant proportion of the variation in ECM species distribution. These effects have usually been investigated independently despite the fact that host trees, acting as ecosystem engineers, may also indirectly shape ECM communities through their ability to modify the abiotic conditions of their environment (Jones et al., 1994; Bennett et al., 2009). The magnitude and direction of these changes are species-specific, and they can be mediated by litter quality and biomass, root exudation and nutrient uptake (Gobran et al., 1998; Mitchell et al., 2007). The complex interactions among host plants, environmental conditions and fungal communities are difficult to disentangle and the relative importance of the indirect host effects on this mutualistic relationship remains unclear.

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Oak forests of southern Spain are an important economic and social resource at the same time that they are a hotspot of endemic and relict plant species (Médail and Quézel, 1999; Anonymous, 2005). Despite the well-known dependence of oaks on mycorrhizal fungi, only a limited number of studies have been done on the belowground ECM communities in oak forests (Walker et al., 2005; Buée et al., 2007; Avis et al., 2008), and even fewer have been carried out in areas with seasonally dry Mediterranean-type climates (Richard et al., 2005; Smith et al., 2007; Morris et al., 2008). In this study we aimed to investigate the importance of the indirect effects of host tree species on the ECM community. For that purpose we studied the ECM fungal community on the roots of two coexisting oak trees, the sclerophyllous evergreen *Quercus suber* (cork oak) and the winter-deciduous *Quercus canariensis* (Algerian oak), under the Mediterranean-type climate of southern Spain, using PCR-based molecular methods.

The research objectives of this study were: i) to assess the diversity and structure of the ECM communities on the roots of these two co-occurring *Quercus* species in two Mediterranean mixed-oak forests, using DNA-based identification techniques; ii) to analyse the relationships between the distribution of ECM species and the measured litter and soil variables by using multivariate methods; iii) to evaluate the extent to which the composition and diversity of the ECM community may be explained by the soil conditions, the host identity and the litter-mediated changes in the topsoil environment (soil and litter) and iv) to compare among several alternative causal models explaining the plant–soil–fungal interactions, in order to test the hypothesis that the studied oak host species, through the differences in their leaf fall quality, may produce key changes in the litter and topsoil chemical composition that in turn, may affect the ECM community assemblage.

2. Materials and methods

2.1. Study area and forest sites

The study area is located in the Aljibe Mountains, in the south of Spain. The region has a rough topography, with the highest peak reaching 1092 m a.s.l. The bedrock dominated by Oligo-Miocene sandstone originates acidic, nutrient-poor soils (*Palexeralfs*; Soil Survey Staff, 2006) frequently interspersed with layers of marl sediments that yield soils richer in clay (*Haploxererts*; Soil Survey Staff, 2006). The climate is of the sub-humid Mediterranean-type with most rainfall (95%) occurring from October to May. See detailed descriptions of the area in Ojeda et al. (2000), and Anonymous (2005).

The vegetation is dominated by evergreen cork oak (*Q. suber* L.), mixed with the winter-deciduous Algerian oak (*Q. canariensis* Willd.), which is locally abundant in the valley bottoms (Urbieta et al., 2008). The arborescent shrubs *Phillyrea latifolia* L. and *Pistacia lentiscus* L. dominated the understorey. The area has been protected since 1989 as “Los Alcornocales” (meaning "the cork oak forests") Natural Park (Anonymous, 2005).

Two structurally different forest sites, 40 km apart, were selected within the study area. The site at San Carlos del Tiradero (36° 9′ 46″ N; 5° 35′ 39″ W), hereafter called “Forest”, was located in the south of the Park near the coast at 335–360 m a.s.l. on a NE facing slope. The mean annual rainfall is 964 mm, and the mean annual air temperature is 16.6 °C, with a minimum of 4.1 °C. The Forest stand had a high density of trees (769 stems ha^{-1}) with a basal area of 47 $m^2\,ha^{-1}$ (estimated on trees with dbh $\geq$ 1.6 cm).

The other site, at La Sauceda (36°31′54″N; 5°34′29″W), hereafter called “Woodland”, was located inland, in the north of the Park, at 530–560 m a.s.l. on a NW facing slope. It has a mean annual temperature of 15.5 °C, with a minimum of 1.8 °C and a mean annual rainfall of 1470 mm. The Woodland tree density was relatively low with 219 stems ha^{-1} and a basal area of 22 $m^2\,ha^{-1}$ (Pérez-Ramos et al., 2008).

2.2. Sampling design

At each forest site (f: Forest, w: Woodland), six adult individuals of *Q. suber* (S) and six of *Q. canariensis* (C) located in a matrix of coexisting oak species and spread across approximately 1 ha were selected. Thus a total of 24 oaks were sampled that can be grouped into four categories (Cf, Cw, Sf, Sw; each with six replicates) of two combined factors: oak species and forest site. The selected trees were estimated to be more than 50 years old.

Leaf fall, litter, topsoil ($\sim$1400 cm^3, 0–25 cm depth) and subsoil ($\sim$1400 cm^3, 25–50 cm depth) were sampled beneath the canopy of each selected oak at approximately 2 m from the trunk in November 2006. Annual leaf fall was collected by four traps (50 cm diameter) located under each tree. The contents were removed, and the leaves were separated and dried. Two 30 × 30 cm quadrats were sampled to assess the litter biomass, by the harvesting and drying method (expressed as kg dry mass m^{-2}). Both leaf fall and litter samples were composed by leaves from one oak species since the closest neighbours of the selected trees were individuals of the same species and they had no significant understorey cover under their canopy. Once the litter layer was removed, cores of soil were extracted with a cylindrical auger; four samples of topsoil (0–25 cm) and four of subsoil (25–50 cm) were taken under each oak tree in the four cardinal directions and pooled into single representative samples.

Superficial roots ($\sim$0–15 cm depth) approximately equal in length ($\approx$20 cm) were taken from each selected tree, close to the litter and soil sampling points, in November 2007. Root samples were kept moist in sealed plastic bags and transported inside an ice-box to the laboratory, where they were stored at 4 °C. Within two days, each root system was examined under a binocular microscope, and 20–22 ectomycorrhizal root tips from each tree were randomly picked free of debris, removed with tweezers and individually stored frozen in 100 μl of 2× CTAB buffer.

2.3. Leaf fall, litter and soil analyses

Samples of leaf fall and litter were dried at 70 °C, weighed, and ground for chemical analysis. Soil samples were dried (30 °C for 48–72 h) and crushed to pass through a 2 mm sieve. Soil acidity (pH) was determined potentiometrically in a 1:2.5 soil:H_2O solution. The percentage of soil carbon was estimated using a Total Organic Carbon Analyzer (TOC-V_{esh}). The available P was estimated using the Bray 1method (Bray and Kurtz, 1945). The total concentration of several macro-nutrients (Ca, K, Mg, P and S) in plant tissues and soils was determined by acid digestion with nitric acid or *aqua regia* followed by ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry). Plant and soil nitrogen was determined by using Kjeldahl digestion and subsequent distillation–titration in a Bran-Luebbe Autoanalyzer. See methods in Allen (1989).

2.4. Ectomycorrhizal DNA extraction, amplification and sequencing

From each ectomycorrhiza sampled, DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, Charbonnieres, France). The CTAB extraction buffer was removed, and the ectomycorrhizas were rinsed with sterile water. Two hundred microlitres of Nuclei Lysis Solution were added. Samples were ground into slurry using a micro-homogeniser with sterilised tips and then incubated for 15 min at 65 °C. Subsequently, 67 μl of protein precipitation