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Is the potential for the formation of common mycorrhizal networks influenced by fire frequency?

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ABSTRACT

We investigated the influence of fire return interval length on the ectomycorrhizal (ECM) community of a *Pinus pinaster* dominated forest and on the potential for common ECM networks (CMNs) between understorey shrubs and *P. pinaster*. ECM root tips were sampled from five shrub species belonging to the genera *Arbutus*, *Cistus* and *Halimium* and from maritime pine in four areas of central Portugal characterized by differing fire return interval length. Fungal symbionts were identified using molecular techniques with direct sequencing of the nrDNA ITS region.

Twenty nine ECM species and sixteen non-ECM root inhabitants were identified. Six years after wildfire disturbance ECM species richness did not differ significantly between unburnt and burnt areas. Nine ECM fungal species were common to pine and shrubs and both their frequency of occurrence and proportion were significantly higher in the unburnt area when compared with both areas subjected to fire.

Our study revealed that while the potential for CMNs between understorey shrub species and pine seemed to be maintained in the long fire return interval area, recurrent fires significantly reduced the frequency of occurrence and the proportion of common symbiont species. High fire frequencies could therefore delay the process of re-colonization by pine seedlings limiting their dispersal in new settings.

1. Introduction

Most ectomycorrhizal (ECM) fungi are not host specific and can simultaneously colonize multiple plant species including canopy trees and understorey plants (Bruns et al., 2002). Thus, plants benefit from the association with a large variety of ECM fungal species that may facilitate access to additional nutrient pools and increase the probabilities that seedlings will not be limited after dispersal in new settings (Kennedy et al., 2003; Ishida et al., 2007; Nara, 2006; Smith et al., 2009). Non-specific ECM fungi have the potential to form common mycorrhizal networks (CMNs) that connect individual plants from the same or different species. CMNs mediate the coexistence of plant species since they mediate plant competition (Perry et al., 1989; Kytöviita et al. 2003), allow nutrient and/or water sharing between linked individuals (Finlay and Read, 1986a,b; Simard et al., 1997b), prevent nutrient leaching, and facilitate seedling establishment (Richard et al., 2009; van der Heijden and Horton, 2009).

The presence of ECM fungi on early colonists is important for facilitating the establishment of seedlings of late-successional woody plant species (Nara and Hogetsu, 2004), providing compatible fungal inoculum even to phylogenetically distant plant individuals as occurs in primary succession (Nara, 2006). Horton et al. (1999) showed that the ECM fungi associated with *Arctostaphylos uva-ursi* contribute to *Pseudotsuga menziesii* seedling establishment in the chaparral from the central coast of California. Recent research has also shown that shrubs associated with ECM fungi (*Arbutus* and *Helianthemum* spp.) favor the colonization of neighboring tree seedlings (*Quercus* spp.) during shrubland-forest succession in Mediterranean ecosystems (Dickie et al., 2004; Richard et al., 2005).

The existence of compatible ECM fungal symbionts is also essential for the post-disturbance recovery of plant communities dominated by "ecologically obligate" ECM hosts, such as most

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forests of temperate and Mediterranean regions (Smith and Read, 1997). Non-specific ECM fungi may help to recover forest communities after disturbance events, such as wildfires, by associating with both pre- and post-disturbance hosts (Perry et al., 1989). Understorey shrubs could therefore play a major role in the recovery of the ecosystem after a disturbance event acting as a bridge between the pre-fire ECM fungal communities and emerging seedlings (Perry et al., 1989). However, repeated wildfires might hinder the regeneration of the original plant guild and compromise the functioning of the ecosystem by leading to the regression of vegetation (Pignatti, 1995; Pausas et al., 2004; Rodrigo et al., 2004) and modifying the structure and composition of soil fungal communities (Buscardo, 2011). Most previous studies have considered the effects of single wildfire events (Baar et al., 1999; Stendell et al., 1999; Grogan et al., 2000; Smith et al., 2004; Buscardo et al., 2011) or repeated prescribed burning (Tuininga and Dighton, 2004; Hart et al., 2005; Bastias et al., 2006; Anderson et al., 2007) on soil fungal communities. From these studies emerged that ECM root biomass can be reduced by repeated burning and that changes to belowground ECM fungal community structure are more pronounced with more frequent burning. Changes in the fire regime may reduce ecosystem resilience (Díaz-Delgado et al., 2002), increase erosion phenomena, and lead to loss of plant cover and soil nutrient impoverishment (Buscardo, 2011). However, while it is known that single wildfire events induce an increase in the proportion of non host-specific ECM species (Jones et al., 1997; Simard et al., 1997a; Horton and Bruns, 1998) we do not have information about the consequences of repeated wildfires on the structure and composition of these species.

With this study we wanted to determine the identity of ECM species associated with maritime pine and five co-occurring understorey shrub species in areas affected by different fire regimes. Our objectives were (i) to characterize the ECM fungal communities associated with those species in four areas with distinct fire frequency (measured as fire return interval length) and (ii) to estimate the influence of fire frequency on the potential for common ECM networks between understorey shrub species and *Pinus pinaster* Ait.

Single wildfire events allow natural pine regeneration in the study area, while recurrent wildfires hinder it completely, eliminate living pine roots and associated ECM hyphae and alter both above- and belowground ecosystem structure (Buscardo, 2011). Under these circumstances, we hypothesized a reduction of common symbionts between understorey shrub species and maritime pine in the short fire return interval area.

2. Materials and methods

2.1. Study sites

The study area is located in Central Portugal between Alvito da Beira (39°48′N, 7°49′W, altitude 500–600 m) and Isna de Oleiros (39°51′N, 7°51′W, altitude 750–850 m). The region is characterized by a Mediterranean climate with hot dry summers and cool wet winters, a lithosol soil and a plant community dominated by *P. pinaster* prior to fire disturbance.

Sampling was performed in areas with different fire histories that represent the fire return interval lengths in the region during the last 30 years (Pereira et al., 2006): unburnt (not affected by wildfire in the last 40 years), burnt with long fire return interval (average of fire return interval of approximately 24 years and last affected by wildfire in 2003), and burnt with short fire return interval (average of fire return of 7.6 years affected by fire in 1992 and 2003). While long fire return interval length allowed the typical autosuccession response of Mediterranean vegetation ecosystems, short fire return interval length hindered pine regeneration.

The unburnt areas (UB) are characterized by an open uneven-aged maritime pine forest (dominant trees with trunk diameters of 40–45 cm) with an understory shrub community dominated by *Erica* spp., *Halimium* spp. and *Pterospartum tridentatum* (L.) Willk. The burnt area with a long fire return interval (B) shows vigorous natural pine regeneration with the same shrubby species as present in UB area. Two different types of vegetation can be found in the areas affected by recurrent fires (short fire return intervals): shrublands dominated by *Cistus* spp. with a scattered distribution of *Arbutus unedo* L. (B1), or shrublands composed of *P. tridentatum*, *Erica* spp. and *Halimium* spp. (B2). Including samples from these two types of shrubland allow to assess possible influences of vegetation composition on the ECM fungi present in the respective arrested stages of succession.

2.2. Soil sampling and analysis

In June 2009, nine soil samples were collected at each site at a depth of approximately 20 cm after removing litter using a shovel. Soil samples were subsequently pooled per site, homogenized and cleared from debris using a 2 mm sieve. Soils were analyzed for organic matter (OM), pH (H_2O), P_2O_5, K_2O, Ca^{2+}, Mg^{2+} and total N at the soil laboratory of the Coimbra Higher School of Agriculture. In November 2009, soil blocks of 12×12 cm^2 surface by 20 cm depth were also collected from 8 individuals of each studied plant species, along a transect of approximately 600 m in UB, B, B1, and B2 (see Table 1). A total of 80 soil samples were collected for further analyses.

Roots were extracted from each soil sample in the laboratory, gently washed, examined for mycorrhizal infection under a dissecting microscope and considered to be ECM if root hairs were absent, if they appeared swollen, and in presence of hyphae or hyphal sheath. For each soil sample, all mycorrhizal roots were sorted into morphotypes based on their colour, size, texture, emanating hyphae and rhizomorphs, and distinct branching morphology (Agerer, 1991). 10 out of the 80 samples did not contain roots with ECM root tips and were discarded from further analysis. All mycorrhizal root tips from the remaining 70 samples were morphotyped and the percentage of root colonization was recorded for each morphotype. Single ECM root tips, one to six of each morphotype per soil core, rendering a total of 265 analyzed samples, were subjected to DNA extraction and PCR amplification of the nrDNA internal transcribed spacer (ITS) region as described in Buscardo et al. (2010). Single PCR products were purified from agarose gel using QIAquick gel PCR purification kit (QIAGEN) and sequenced at Macrogen laboratories (South Korea).

Although soil samples were collected underneath plants of each species, host misidentification was a risk due to the possibility of having roots of different plant species in the soil sample. To avoid

Table 1
Plant species sampled in four areas with different fire regimes. UB, unburnt; B, long fire return interval; B1, short fire return interval, dominated by *Cistus ladanifer*; B2, short fire return interval, dominated by ericaceous shrubs, *Halimium* spp. and *Pterospartum tridentatum*.

	UB	B	B1	B2
Pinus pinaster Ait.	x	x		
Halimium ocymoides (Lam.) Willk.	x	x		x
H. lasianthum (Lam.) Spach *subsp. alyssoides* (Lam.) Greuter & Burdet		x		x
Arbutus unedo L.			x	
Cistus ladanifer L.			x	
Cistus laurifolius L.			x	