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Structure and phylogenetic diversity of post-fire ectomycorrhizal communities of maritime pine

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Abstract Environmental disturbances define the diversity and assemblage of species, affecting the functioning of ecosystems. Fire is a major disturbance of Mediterranean pine forests. Pines are highly dependent on the ectomycorrhizal (EM) fungal symbiosis, which is critical for tree recruitment under primary succession. To determine the effects of time since fire on the structure and recovery of EM fungal communities, we surveyed the young *Pinus pinaster* regenerate in three sites differing in the elapsed time after the last fire event. Pine roots were collected, and EM fungi characterized by sequencing the internal transcribed spacer (ITS) and the large subunit (LSU) regions of the nuclear ribosomal (nr)-DNA. The effects of the elapsed time after fire on the EM community structure (richness, presence/absence of fungi, phylogenetic diversity) and on soil properties were analysed.

Fungal richness decreased with the elapsed time since the fire; although, the phylogenetic diversity of the EM community increased. Soil properties were different depending on the elapsed time after fire and particularly, the organic matter, carbon-to-nitrogen (C/N) ratio, nitrogen and iron significantly correlated with the assemblage of fungal species. Ascomycetes, particularly *Tuberaceae* and *Pezizales*, were significantly over-represented on saplings in the burned site. On seedlings, a significant over-representation of *Rhizopogonaceae* and *Atheliaceae* was observed in the most recently burned site, while other fungi (i.e. *Cortinariaceae*) were significantly under-represented. Our results are consistent with the hypothesis that fire can act as a selective agent by printing a phylogenetic signal on the EM fungal communities associated with naturally regenerated pines, pointing out to some groups as potential fire-adapted fungi.

Keywords Ectomycorrhizas · Fire ecology · Fungal diversity · *Pinus pinaster* · Phylogenetic structure

Introduction

Fire is an integral disturbance of Mediterranean forest ecosystems (Keeley et al. 2012). Wildfires generate drastic spatial-temporal changes that usually reduce landscape heterogeneity and affect the distribution and functioning of all living organisms (Loepfe et al. 2010). Expected drought and longer fire seasons related to climate change have led to predictions of increased wildfire activity (frequency, intensity and duration) (Thompson and Calkin 2011), which represent a major threat to vulnerable ecosystems such as the Mediterranean one.

Mediterranean plants have evolved fire-adaptive strategies that partially explain the high resilience of Mediterranean forest ecosystems (Keeley et al. 2012). However, post-fire natural regeneration of forests depends on multiple factors such as the initial plant species composition, fire intensity, seed bank availability, and the recovery of soil microbial communities (Rodrigo et al. 2004; Hart et al. 2005; Buscardo et al. 2011). Mediterranean pines display remarkable colonization abilities as a result of both high genetic variability and phenotypic plasticity. *Pinus pinaster* Ait. is a good example of a widespread tree species in the Mediterranean basin showing fire-adaptive traits such as serotiny or thick bark (Tapias et al.

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2004). Successful pine recruitment after a fire can be hindered by the adverse post-fire environmental conditions, the competition with neighbouring plants and the availability of mycorrhizal fungi in soil (Rodrigo et al. 2004; Dickie et al. 2009; Rincón and Pueyo, 2010; Buscardo et al. 2011). EM fungi play major roles in the functioning of forest ecosystems notably by improving the uptake and transfer of nutrients and water to the host plants, so decreasing their moisture and nutritional stresses especially under adverse environmental conditions (Smith and Read 1997). Since pine species are obligatory mycorrhizal, post-fire-regenerated seedlings are highly dependent on the availability of active EM fungal propagules in soil (i.e. mycelium, spores) to establish the symbiotic partnership that will help them to cope with the adverse environmental conditions imposed by fire (Dahlberg et al. 2001; Buscardo et al. 2011).

Fire alters soil properties and reduces the microbial activity, causing deep changes on the availability of nutrients and directly impacting the vegetation recovery (McMullan-Fisher et al. 2011). Particularly, EM fungi are affected by fire through the increase of soil temperature, the combustion of the organic layer, the deposition of ashes and the soil surface run-off (Cairney and Bastias 2007). Additionally, fire causes the loss of vegetation and thus the lack of colonisable roots, disrupting the continuity of the mycorrhizal interaction and affecting the life cycle of the EM fungi.

Fire generally resets the specific composition and structure of EM fungal communities (Torres and Honrubia 1997; Stendell et al. 1999; Grogan et al. 2000; Dahlberg et al. 2001; Bastias et al. 2006; Rincón and Pueyo 2010), which require many years to return to pre-fire conditions (Treseder et al. 2004; Kipfer et al. 2011). Interactions among fungal species within the community can be determinant of important ecosystem functions such as nutrient cycling (Kennedy 2010). However, at present, the consequences of structural changes of EM communities in terms of ecosystem functioning remain largely unknown (Johnson et al. 2012). Phenotypic differences or similarities among species within a community are founded in their evolutionary history, and it is commonly accepted that inter-specific interactions are influenced by the net ecological similarity of taxa, with closely related species tending to be ecologically more similar than distantly related ones (Webb et al. 2002). The phylogenetic analysis of diversity can thus be a useful indicator for estimating the relative importance of evolutionary and ecological forces in shaping living communities (Webb et al. 2002; Pausas and Verdú 2010). How these assumptions apply to microorganisms (Horner-Devine and Bohannan 2006; Maherali and Klironomos 2007) and, in particular, to EM fungal communities (Branco and Ree 2010; Peay et al. 2010) has been little explored.

The aim of this study was to determine differences in the structure and recovery of EM fungal communities associated with naturally regenerated pines in three sites differing in the elapsed time since the last fire event. It was hypothesized that time since fire will modify the structure of the EM community by printing a phylogenetic signal that will allow the identification of fire-adapted fungi (Baar et al. 1999, Claridge et al. 2009). The elapsed time after fire was thus predicted to alter not only the richness of the EM community but also its phylogenetic diversity. The fire-related response of the EM community was expected to be linked to soil changes, and differentially expressed depending on the age of the regenerated host. Gaining knowledge on the structure of post-fire EM fungal communities will contribute to our understanding of the complex temporal-spatial feedbacks between soil-fungus-plant interactions and of the potential recovery of these communities after disturbance.

Materials and methods

Site description

The study was conducted in a *P. pinaster* forest located in Guadalajara, central-east Spain (41º3′N, 2º26′W), where three sites in a fire chronosequence were defined. The study location was at approximately 1,000 m elevation and had Mediterranean climate with cold, wet winters and hot, dry summers. Mean monthly temperatures range form −4 to 28 °C and annual precipitation is of 580 mm, most falling between April and June. Soils are brown and formed by parent ferruginous sandstones and siliceous puddingstones from the Buntsandstein.

In the study location, two neighbouring areas were burned in stand replacing wildfires in the summers of 1994 (Fi-94) and 2005 (Fi-05). A control site was established in an adjacent *P. pinaster* forest unaffected by fire at least in the preceding 60 years (dominant trees of approximately 40-cm diameter) (Fig.S1). Fire-prone Mediterranean pines such as *P. pinaster* usually show fire-adaptive traits (i.e. serotiny and fire-induced seed germination) enabling a quick and efficient post-fire regeneration, as it was the case in all the sites chosen for this study. In the study locations, plant communities represented several stages of secondary succession; the plant community in Fi-05 was dominated by herbaceous perennials with low cover of small-sized shrubs (i.e. *Lavandula stoechas* Lam., *Thymus* sp., *Santolina* sp.), scattered 3-year-old *P. pinaster* seedlings and none adult pines. In Fi-94, it consisted of dispersed evergreen shrubs (i.e. *Cistus laurifolius* L., *Rosa canina* L.), conspicuous *P. pinaster* saplings and seedlings, herbaceous perennials and isolated saplings of *Quercus pyrenaica* Willd. and *Quercus ilex* L. In the control site, forest over-storey was dominated by *P. pinaster*, the mid-storey was primarily composed of small trees of *P. pinaster* and isolated *Q. pyrenaica* and under-storey composed of evergreen shrubs (i.e. *C. laurifolius* L., *Cytisus scoparius* L. (Link)), lichens and mosses.

Experimental design and sampling

Three different sites in *P. pinaster* which stand within an area of approximately 40 km^2 were selected according to the time since the last fire event: (a) greater than 60 years after fire (Control), (b) 1994 burn, 14 years after fire (Fi-94) and (c) 2005 burn, 3 years after fire (Fi-05) (Fig. S1). At each site, three replicated 100×100 m plots separated more than approximately 1 km were selected in a haphazard fashion for sampling roots, in spring 2008. The study was focused on early regeneration, considering young pines with the expected age similar to that of having been naturally regenerated after the burns of 2005 and 1994 (3 and 14 years, respectively). Two kinds of samples were harvested in a haphazard fashion for root-tip collection: (1) roots of seedlings (3-year-old pines) in all sites (control, Fi-94 and Fi-05), and (2) roots of saplings (14-year-old pines) in control and Fi-94 sites. In the first case, five to eight individual seedlings per plot were carefully excavated in each site. Roots of saplings were excavated in a 10×10-cm surface and 20 cm of soil depth at 0.5 to 1 m from the trunk in north and south directions, traced back to the target sapling, and a total of five samples per plot were collected in each site. The number of pine internodes was counted for age estimation. In all cases, samples were separated by a minimum of 5 m.

Processing of EM samples and soil analyses

Once collected, roots were removed from soil by hand, gently washed with water over 2 and 0.5 mm sieves, and cut into 2- to 3-cm segments. Root segments were observed under a dissecting microscope, and a subset of approximately 200 randomly selected root tips per sample was taken. Root tips were classified as mycorrhizal based on the existence of fungal mantle and mycelium, or non-mycorrhizal based on their absence and the existence of root hairs. Total percentage of EM roots per sample (number of mycorrhizal tips / total number of root tips) was assessed and EM tips were classified by morphotypes based on the characteristics of their mantle and extra-matrical mycelium (branching, surface colour, texture, emanating hyphae and rhizomorphs; Agerer 1987–1998). When ectomycorrhizas were not evident, cross-sections were made and examined under the microscope to verify the presence of the Hartig net. For each sample, three to five EM root tips of each morphotype were separately collected and stored at −80 °C for molecular analyses. Soils from each sample were air dried, sieved to 2 mm and measured for different variables: pH (1:5*w*/*v* in H_2O), electrical conductivity (1:5*w*/*v* in H_2O), organic matter (OM) and total C, total N (determined by the Kjeldahl method), and extractable K, Mg, Mn and Fe determined by inductively coupled plasma spectrometry (Optima 4300DV, Perkin-Elmer).

Molecular identification of EM fungi

To identify each EM fungal morphotype in a molecular basis, the ITS region of nuclear ribosomal DNA was analysed by combining restriction fragment length polymorphism (RFLP) and sequencing analyses. DNA was extracted from three root tips of each morphotype in every sample as described by Gardes and Bruns (1993). Polymerase chain reaction (PCR) amplifications were done using the primer pairs ITS-1F/ITS-4 or ITS-1F/ITS4B under the PCR conditions previously described (White et al. 1990; Gardes and Bruns 1993). Negative controls without DNA were performed to detect possible contaminations. ITS-PCR products were separately digested by the endonucleases *Hinf*I, *Msp*I and *Taq*I (NewEngland Biolabs, UK) and the restriction patterns (RFLPs) were analysed by electrophoresis (5 V/cm) in 2.5 % low-fusion-point agarose gel (MS4, Pronadisa, Spain) in TAE buffer providing a resolution of approximately 10 bp. Samples were scored for the presence/absence of the fragments obtained by each endonuclease and a binary matrix was generated. Jaccard similarity indices were calculated for each pair of samples, and a dendrogram was constructed by the un-weighted pair group method of analysis using the SPSS v 17.0 software. Samples were distributed within clusters on the basis of their RFLP patterns, which allowed verifying and correcting the previous classification of morphotypes made by morphological features. Samples within a same RFLP cluster (95 to 100 % similarity) were pooled and classed as a separate EM type, and a sample of each unique RFLP pattern was chosen for sequencing. PCR products were purified using the GFX™ PCR Gel Band Purification Kit (Amersham Pharmacia Biotech, USA), before sequencing. Sequencing reactions were performed using the ABI PRISM™ Dye Terminator Cycle Sequencing Kit (Applied Biosystems, USA) with the primers ITS-1F/ITS-4, and analysed in an automatic ABI PRISM 3730 sequencer (Applied Biosystems).

Forward and reverse sequences were aligned and manually corrected, and pair-wise comparisons were made between all sequences, which were then clustered based on the percentages of similarity. Taking into account the different sources of expected variation when assembling fungal ITS sequences into clusters, 97 % or greater sequence similarity was considered to define a unique sequence type or fungal operational taxonomic unit (OTU) (Nilsson et al. 2008). Tentative taxonomic identifications to the genus or species level of each unique OTU were performed by basic local alignment search tool (BLAST) searching in the GenBank and UNITE databases, based on sequence similarity with ITS sequences only of voucher sporocarps. Proper species matches were considered if a minimum overlap of 450 base pairs and 97 % or greater similarity to an ITS sequence in the Genbank or UNITE databases were obtained. When no taxonomic identification was possible, the sample was classed as "unknown"

EM-#. Additionally, partial amplification of the 25S nuclear ribosomal LSU gene was carried out for each OTU using the primer pairs LROR/TW13 and ctb6/TW13, as described by Tedersoo et al. (2006). All LSU sequences were queried by BLAST, their previous taxonomic identification verified, and used to perform the phylogenetic analysis. Representative ITS and LSU sequences of each fungal OTU were deposited in the GenBank database (accession numbers JQ975951-JQ976017) (Table S1).

Phylogenetic analysis

A phyogenetic tree was built using the 5.8S and 25S LSU sequences of all EM fungal OTUs (Fig. S1). A sequence of *Glomus mosseae* (FN547492) was used as out-group. Alignments were built with the MUSCLE v3.6 (Edgar 2004) and manually edited with the BioEdit v7.0.9. Ambiguously aligned regions were removed using the Gblocks 0.91b (Castresana 2000). The phylogenetic tree was constructed with the BEAST v 1.6.1 assuming a general time-reversible model of nucleotide substitution rates, a speciation Yule process and an uncorrelated relaxed lognormal clock (Drummond and Rambaut 2007). Topological constraints following different phylogenetic studies of the kingdom Fungi were applied (Hibbet 2006; James et al. 2006; Moncalvo et al. 2006; Sugiyama et al. 2006; Schoch et al. 2009). A chronological constraint of 390±50 million years was applied to the split between Basidiomycetes and Ascomycetes (Berbee and Taylor 1993). A maximum clade credibility tree was summarized from the trees resulting after MCMC sampling every 10^3 generations over a total of 10^7 steps (Fig. S2).

Statistical analyses

Relative frequency was defined as the absolute frequency of an individual OTU (the number of samples on which it was found divided by the total number of samples) divided by the sum of the absolute frequencies of all OTUs. Similarly, relative abundance (root tip number) was estimated as the absolute abundance of an individual OTU (sum of abundances of a given OTU divided by the total number of samples) divided by the sum of the absolute abundances of all fungal OTUs. The effects of the elapsed time after fire on EM fungal general occurrence and assemblage patterns (i.e. relative frequency/abundance, the presence/absence of OTUs) were tested considering all data. Richness and phylogenetic structure of the EM community were independently analysed for seedlings and saplings to separate potential host age-related effects.

The number of fungi per sample and fungal richness (S) were determined for seedlings and saplings, and the Shannon-Wiener diversity index (H') and dominance (Simpson index D) calculated (Magurran 1988). A generalised linear model either based on Gaussian (EM colonization, Shannon diversity and dominance) or a Poisson (fungi per sample and richness) error distribution, using the time since fire as ordered factor (Fi94-Fi05-Control), was used for testing linearized and quadratic contrasts. Significant linear contrasts ($P<0,05$) with positive or negative t values indicate, respectively, an increase or decrease of the dependent variable in response to the factor. Significant quadratic contrasts with positive or negative t values indicate respectively that the dependent variable reaches minimum or maximum values at the intermediate treatment of the rank.

The effect of the elapsed time after fire on the soil variables was analysed by analysis of variance, and significant differences among treatments were separated by Tukey's test ($P<0.05$). The effect of the elapsed time after fire on the assemblage of fungal OTUs was tested by nonparametric multivariate analysis of variance (ADONIS) using the Raup–Crick similarity index, a probabilistic measure based on the presence/absence of data, and statistical significance tested against 10,000 null permutations. The EM community structure was drawn by non-multidimensional scaling (NMDS) using the presence/absence of data of fungal OTUs, and fungal-environmental correlations, tested against 10,000 null permutations, were plotted onto the ordination by using the *envfit* R function of MASS R package. These analyses were performed as implemented in the vegan and MASS R packages (Oksanen et al. 2011).

The phylogeny obtained was used to calculate the net relatedness index (NRI) using the program Phylocom (Webb et al. 2008). NRI is an indicator of phylogenetic diversity, which measures the mean pair-wise phylogenetic distance between all taxa within a sample standardized against a null model. In this case, the null model used sampled at random two species from the phylogenetic tree. Results were consistent with other null models available in the *picante* R package (Kembel et al. 2010). The effect of the elapsed time after fire on the phylogenetic diversity (NRI) of EM fungal communities of seedlings and saplings was analysed by a generalised linear model based on Gaussian error distribution, using the time since fire as an ordered factor (ranked as Fi-94-Fi-05-Control) for testing linearized and quadratic contrasts ($P<0.05$), as previously explained. To identify the phylogenetic clades that were significantly influenced by the time since fire or by the age of the tree, the procedure *nodesig* in Phylocom was used (Webb et al. 2008). This procedure tests whether a particular node in the phylogeny has significantly more or less descendent taxa in a sample than predicted by a null model. The null model randomly draws s taxa from the phylogenetic tree, where s is the number of taxa in the sample (Webb et al. 2008). All the analyses were performed with the R software v.2.15.1 (R Development Core Team, 2011).

Results

Occurrence and identification of ectomycorrhizal fungi

At the control site, most samples harboured three or four different EM fungal OTUs with a maximum of five, whereas in the burnt sites the majority of samples harboured only one (Fi-05) or two (Fi-94) different fungi (Fig. 1a). The frequency curves showed the EM fungal communities represented by few dominant fungi and many less frequent ones, particularly in both burned sites (Fig. 1b).

A total of 67 different EM fungal OTUs were identified in the study, among which 32 were found only in one sample (Table S1). Thirty-seven different OTUs were respectively identified in the control and the Fi-94 sites, and 20 in the most recently burned site. The 75 % of OTUs were Basidiomycetes and the 25 % Ascomycetes (Table S1). The BLAST search resulted in the tentative identification of 38 % of the EM OTUs to the level of species, 18 % to genus and 28 % to family, while 16 % remained unidentified (Table S1).

Only five OTUs were found in all sites: EM-1 (*Atheliaceae*), *Cenococcum geophilum* Fr., *Craterellus tubaeformis* (Fr.) Quél., *Rhizopogon graveolens* (Vittad.) Tul. and C. Tul. and *Russula nauseosa* (Pers.) Fr (Table S2). Across all fire treatments, 41 different fungal OTUs were found on seedlings and 37 on saplings, and the 30 % of the total OTUs were found on both, coincident with the most frequent fungi overall (Table S2). Usually, the most frequent fungi were also the most abundant (Fig. 1c), except in the case of *C. geophilum* that was highly frequent but with low abundance in all sites. Far from the rest, the most frequent and abundant fungus in the burned sites was *R. graveolens* (Fig. 1c; Table S2).

The highest diversity was found for the families *Thelephoraceae* (12 OTUs, including *Tomentella* and *Thelephora*), *Cortinariaceae* (11 OTUs, including *Cortinarius* and *Inocybe*) and *Russulaceae* (10 OTUs, including *Russula* and *Lactarius*) (Tables S1, S2).

Mycorrhization rates and EM fungal richness

Rates of EM root colonization ranged from 63 to 81 % across treatments (Fig. 2a). EM root colonization on seedlings was lowest in site Fi-05, whereas the maximum was reached in the site Fi-94 (Fig. 2a). The percentage of EM root colonization of saplings was not affected by the time since fire (Fig. 2a). On both seedlings and saplings, the number of fungal OTUs found per sample was significantly reduced as the elapsed time since fire was shorter (Fig. 2b). A gradual increase of fungal richness and Shannon diversity coupled with a decrease of dominance were observed as the time elapsed since fire increased, effects that were significant only on saplings (Fig. 2c,d,e).

Effects of time since fire on soil properties and EM fungal assemblages

Soils in Fi-94 showed significant lower values of almost all variables tested compared with soils in the control site (Table 1). In the most recently burned site Fi-05, significant reductions in OM, Fe and Mn contents compared with control soils were also observed (Table 1). The content of nitrogen was significantly reduced in Fi-94 compared with the control, but unaffected in the most recently burned site. When comparing both fire-affected soils that burned 14 years ago had significantly less OM, Fe and Mn than the most recently burned soil, and in both sites, the C/N ratio was significantly reduced compared with the control site (Table 1).

A significant effect among the three sites differentially affected by fire on the presence/absence of EM fungi was shown by nonparametric multivariate analysis of variance ($R^2=0.710$, $P<0.025$). The results were drawn by ordination analysis, which also showed the weight of the edaphic variables in grouping the EM OTUs (Fig. 3). Among soil variables, the organic matter ($P=0.001$), C/N ratio ($P=0.005$), nitrogen ($P=0.037$), and Fe ($P=0.019$) were significant explaining the assemblage of EM fungal OTUs (Fig. 3).

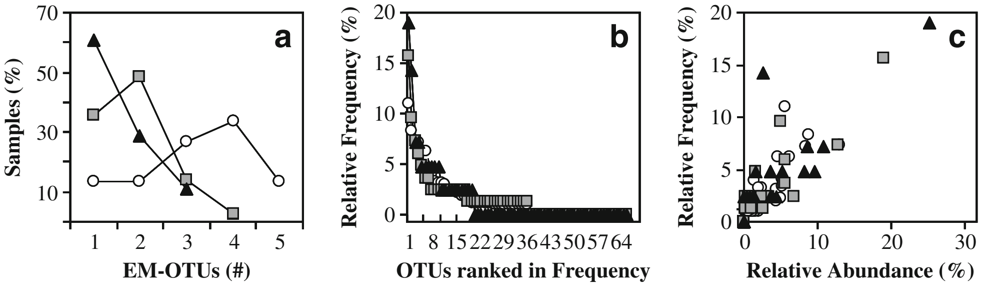

Fig. 1 Occurrence of ectomycorrhizal fungal OTUs associated with naturally regenerated *Pinus pinaster*, in sites differing in the elapsed time since the last fire event: control=greater than 60 years after fire (white circles), Fi-94=14 years after fire (grey squares), and Fi-05=3 years after fire (black triangles). **a** Percentage of samples with a number of fungal operational taxonomic units (OTUs), **b** EM-OTUs ranked by relative frequency and **c** correlation between relative frequency and abundance of EM-OTUs; the three high-frequency outliers correspond to *Cenococcum geophilum*, and the two highest frequency/abundance values to *Rhizopogon graveolens*

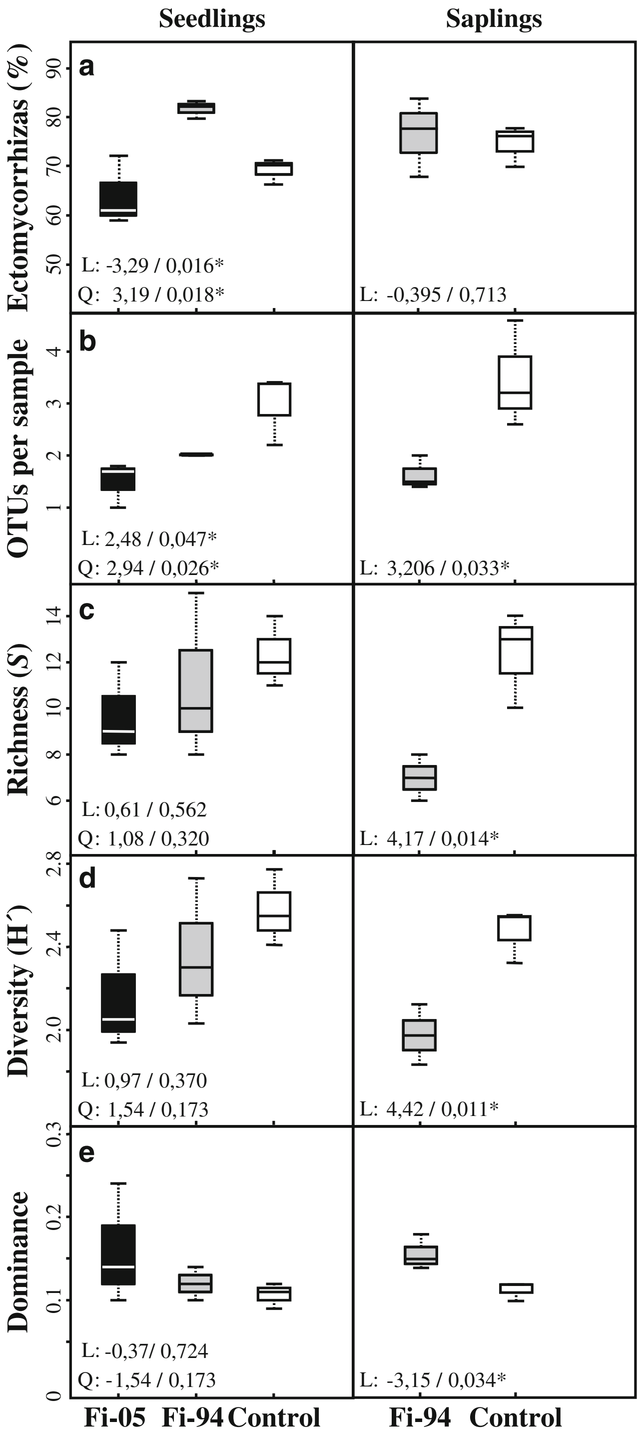

Fig. 2 (**a**) Ectomycorrhizal root colonization, (**b**) number of fungal OTUs per sample and (**c**) richness (S), (d) diversity (*Shannon* index) and (e) dominance of EM fungi associated with roots of *Pinus pinaster* seedlings (left) and saplings (right), in sites differing in the elapsed time since the last fire event: control > 60 years after fire (white), Fi-94 = 14-yr after fire (grey), and Fi-05 = 3-yr after fire (black). Linearized (L) and quadratic (Q) contrasts: t value / P probability (P < 0,05*). Significant L contrasts with positive or negative t-values indicate respectively, an increase or a decrease of the dependent variable in response to the elapsed time after fire. Significant Q contrasts with positive or negative t-values indicate respectively, that the dependent variable reaches minimum or maximum values at the intermediate treatment of the rank (Fi-94/Fi-05/Control)

Table 1 Characteristics of soils collected in three sites differing in the elapsed time after the last fire event: Control = > 60-yr after fire; Fi-94 = 14-yr after fire and Fi-05 = 3-yr after fire. Data = mean ± SE. In each row, different letters denote significant differences among treatments according to Tukey's test (P < 0,05)

Soil variable	Control		Fi-94		Fi-05	
pH	5,5 ± 0,1	a	5,9 ± 0,1	a	5,3 ± 0,2	a
E. Conductivity (μS/cm)	80 ± 4	b	54 ± 2	a	82 ± 4	b
Organic matter (%)	3,6 ± 0,4	c	1,3 ± 0,2	a	2,1 ± 0,2	b
N (‰)	0,75 ± 0,06	b	0,36 ± 0,03	a	0,72 ± 0,08	b
C/N	26,8 ± 0,7	b	17,6 ± 1,3	a	17,5 ± 0,6	a
K (μg/g)	60,0 ± 2,4	a	55,6 ± 2,5	a	52,5 ± 3,2	a
Mg (μg/g)	82,3 ± 4,5	b	58,0 ± 2,5	a	79,4 ± 3,2	ab
Fe (μg/g)	99,4 ± 26	c	46,0 ± 23	a	74,7 ± 29	b
Mn (μg/g)	71,8 ± 6,8	c	11,0 ± 5,2	a	40,1 ± 5,6	b

Phylogenetic diversity of the EM fungal community

In the control site, most samples showed negative NRI values indicating that taxa were phylogenetically clustered (low phylogenetic diversity), whereas in both burned sites, the majority of samples had positive NRI values, indicating phylogenetic overdispersion (high phylogenetic diversity) (Fig. 4). In the case of seedlings, the elapsed time after fire did not significantly affect the phylogenetic diversity of the EM fungal community (Fig 4a). However, clade-level analyses showed that seedlings established immediately after fire (FI-05)

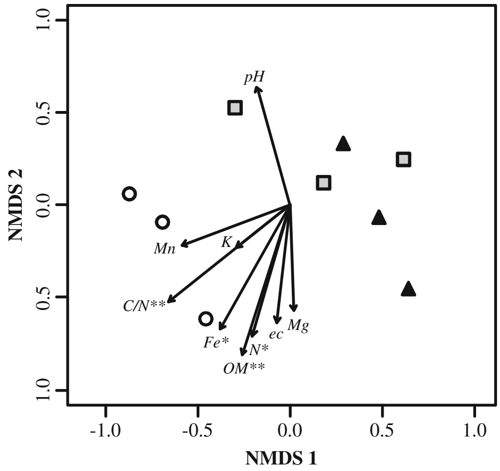

Fig. 3 NMDS analysis of the ectomycorrhizal fungal community associated with naturally regenerated *Pinus pinaster*, in sites differing in the elapsed time since the last fire event: control = > 60 yr after fire (white circles), Fi-94 = 14-yr after fire (grey squares), and Fi-05 = 3-yr after fire (black triangles). Vectors represent the strength and direction of the weight of edaphic variables on affecting the presence/absence of fungal OTUs (P < 0,05*; P < 0,01**). OM = organic matter (P = 0,001); C/N (P = 0,005); N = nitrogen (P = 0,037); pH (P = 0,057); ec = electric conductivity (P = 0,118); K = potassium (P = 0,581); Mg = magnesium (P = 0.198); Fe = iron (P = 0,019); Mn = manganese (0,121)

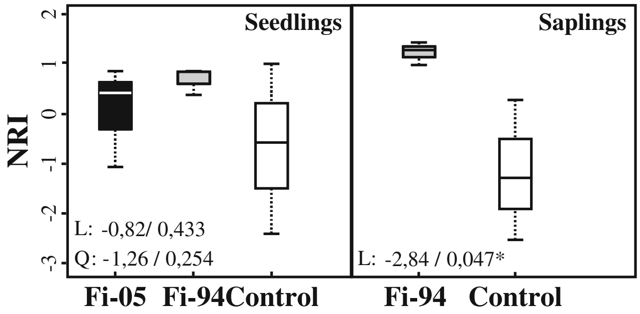

Fig. 4 Phylogenetic diversity (net relatedness index, NRI) of the EM fungal community of *Pinus pinaster* seedlings and saplings, in sites differing in the elapsed time since the last fire event: control = > 60-yr after fire (white), Fi-94 = 14-yr after fire (grey), and Fi-05 = 3-yr after fire (black). Linearized (L) and quadratic (Q) contrasts: t value / P probability (P < 0,05*). Significant L contrasts with negative t-values indicate a decrease of the dependent variable in response to the elapsed time after fire. Significant Q contrasts indicate that the dependent variable reaches minimum or maximum values at the intermediate treatment of the rank (Fi-94/Fi-05/Control)

harboured a significant over-representation of fungi corresponding to *Rhizopogonaceae* and *Atheliaceae*, while other fungi such as *Amanita phalloides* Scr., *Hebeloma cistophilum* Maire, *Laccaria bicolor* (Maire) P.D. Orton, or the clade with OTUs belonging to *Cortinariaceae* were significantly underrepresented (Fig. 5a). This phylogenetic predilection disappeared when seedlings established in the sites with longer elapsed time after fire (Fi-94 and Control, Fig. 5a). In the case of saplings, a significant increase of the phylogenetic diversity of fungal taxa within the EM community was observed in the sites affected by fire (Fig. 4). This increment was probably the consequence of fire significantly reducing the presence of the larger clade of Basidiomycetes and significantly favouring the presence of the clade of Ascomycetes, particularly of the groups Pezizales (*Pezizaceae* and *Pyronemataceae*) and *Tuberaceae* (Fig. 5b).

Discussion

Mycorrhizal colonization was not reduced in the burned sites, and even a significant increase was observed on seedlings in

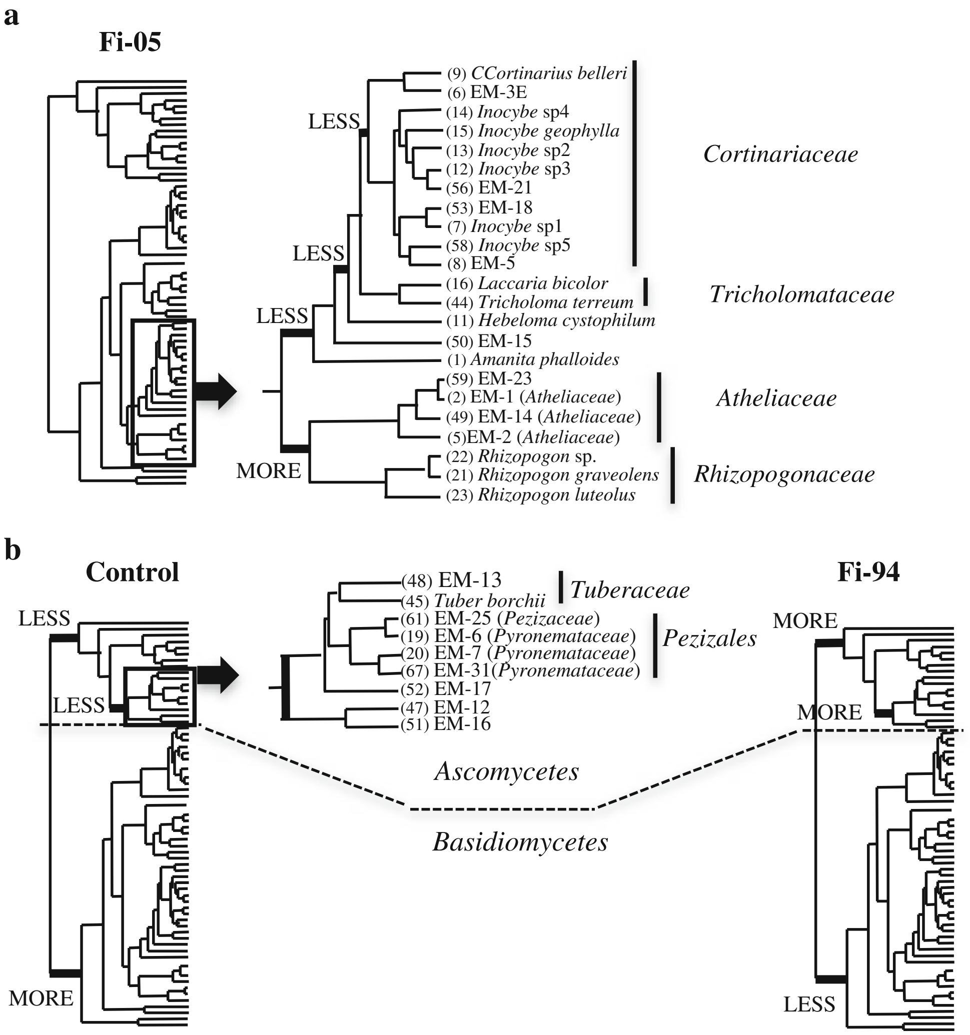

Fig. 5 Phylogenetic structure of the ectomycorrhizal fungal communities of *Pinus pinaster* (a) seedlings and (b) saplings, in sites differing in the elapsed time since the last fire event: Fi-94 = 14-yr after fire, and Fi-05 = 3-yr after fire with respect to the control = > 60 yr after fire. LESS or MORE denote respectively a significant under- or over-representation of that clade in the corresponding treatment of elapsed time since fire, by the *nodesig* Phylocom procedure

the site affected by fire 14 years ago. Negative effects possibly related with fire were observed in fungal richness that was progressively reduced and in dominance that was increased as the elapsed time after fire was shortened, effects that significantly persisted in saplings 14 years after fire. The EM root colonization and fungal richness of post-fire EM fungal communities have been previously reported to be reduced (Dahlberg et al. 2001; Kipfer et al. 2011; LeDuc et al. 2012), or not affected by fire (Jonsson et al. 1999; Grogan et al. 2000; Rincón and Pueyo 2010; Longo et al. 2011) depending on the host tree species, the fire severity, the elapsed time after fire, and the local post-fire edaphic-climatic conditions. Additionally, it should be noticed that due to pseudo-replication, a common limitation of many ecological field studies and particularly of those related with fire (Legendre et al. 2010), effects observed in this study could be attributable to other underlying factors other than fire.

In the most recently burned site Fi-05, seedlings showed the lowest mycorrhizal colonization rates and most samples harboured only one fungal OTU compared with the three or four found per sample at the control site, suggesting that abiotic filtering probably operated through fire-induced reduction of active EM inoculum. Fire can induce a rapid decline of active fungal propagule density, especially in the form of mycelium, by means of soil heating and elimination of host vegetation (Cairney and Bastias 2007). The most direct source of EM inoculum for seedlings installed immediately after fire is the soil bank of fungal spores and other resistant propagules built up over the time (Taylor and Bruns 1999; Buscardo et al. 2010). At subsequent phases of regeneration, fungi from deeper mineral soil layers or propagules from adjacent areas can progressively re-colonize the burned areas (Taylor and Bruns 1999), as it was probably the case in the Fi-94 site, where most of the samples were colonized by two fungi. Interestingly, some fungi found in the control site (i.e. *Inocybe* sp.1, *Rhizopogon* sp., *Russula* sp.1, *Tomentella coerula* or *Tuber borchii*), also appeared in the site affected by fire 14 years ago but not at the most recently burned one, which could be interpreted in terms of re-colonization and ecosystem recovery.

Approximately half of the total fungi were found only in one sample, the majority on seedlings at the site Fi-94, suggesting that point-source inoculum (i.e. spores, isolated mycelial clones) still prevailed especially for the youngest regenerated pines, even 14 years after fire. The viability of potential post-fire EM inoculum sources and the strength of their contribution to fungal re-colonization after fire are processes difficult to determine (Cairney and Bastias 2007).

In our study, changes in the representativeness of some fungal groups were observed in the burned sites, and even the presence or absence of discrete fungi was triggered, shifts that might have been influenced by changes in soil properties (i.e. organic matter, C/N ratio, total nitrogen, Fe). Post-fire chemical and physical changes of the edaphic environment have been previously demonstrated to affect the richness and structure of soil fungal communities (Hart et al. 2005; Cairney and Bastias 2007; LeDuc et al. 2012). In our study, the soils of both burned sites showed significant reductions of organic matter, Fe and Mn, with particularly negative effects probably attributable to fire in the long term (Fi-94 site). After wildfire, organic matter and other soil elements are usually lost by combustion and leaching, losses that can be accentuated over time by runoff events and increased erosion (Certini 2005). The lowest levels of extractable elements (i.e. Mg, Fe and Mn) observed in Fi-94 soils could also be attributed to an indirect effect of pH on their solubility (Pereira et al. 2012). The content of nitrogen was unaffected in the most recently burned soil, but significantly reduced in the soil burned 14 years ago. Fire drives the conversion of organic into inorganic forms of nitrogen that can be more easily lost by volatilization, direct leaching, erosion or denitrification, inducing the reduction of total nitrogen content in soil along the time (Certini 2005). EM fungi are highly conditioned by nitrogen availability (i.e. sporocarp production, root colonization), which can select EM fungi to evolve diverse nitrogen extraction and utilization traits (Avis et al. 2003), and shifts in fungal taxa have been reported to be in a close relation with organic forms of nitrogen (Tweig et al. 2009; LeDuc et al. 2012). In both burned soils, the C/N ratio was considerably reduced 3 and 14 years after fire. This parameter was significant explaining the general EM assemblage pattern. Fire primarily affects soil organic horizons where a high proportion of EM fungi live, inducing higher carbon than nitrogen losses and the reduction of soil C/N ratio, a variable that has been considered a good indicator of fire severity (Pereira et al. 2012). The return of the EM community to a pre-fire structure has been previously related to the progressive gain of soil nitrogen, phosphorous (Avis et al. 2003; Longo et al. 2011) and organic matter and C/N ratio (Treseder et al. 2004; Nilsson et al. 2012), although further studies are needed for better understanding the links among post-fire soil dynamics and the mechanisms governing the fungal assemblage patterns.

A general tendency of low phylogenetic diversity (clustering) of the EM community was observed in the control site, in contrast with the high phylogenetic diversity (over-dispersion) found in both burned sites. Specifically, the phylogenetic diversity of the EM fungal community of saplings at the burnt site was significantly higher compared with the control site. It was attributable to the predominance of Ascomycetes over Basidiomycetes, with a significant over-representation of the clades belonging to *Tuberaceae* and *Pezizales*, fungi that can produce high amounts of resistant spores. The increase of Ascomycetes has been usually reported in the EM communities at early stages of secondary succession (Torres and Honrubia 1997; Grogan et al. 2000; Cairney and Bastias 2007; Claridge et al. 2009; Rincón and Pueyo 2010; Buscardo et al. 2010).

Immediately after fire, some ascomycetous fungi (i.e. saprophytic *Geopyxis carbonaria* or mycorrhizal *Peziza violacea*) produce intense fruiting flushes and subsequent spore accumulation, probably as a fire-adaptive strategy to better exploit carbonised resources and/or competitively colonize the roots of newly regenerated plants. Competition has been invoked to increase the phylogenetic diversity in ecological communities (Webb et al. 2002). More specifically, competition through inhibitory priority effects has been shown to produce an increase of phylogenetic diversity in nectar yeast communities because the negative effect of early-arriving on late-arriving species is stronger between closer relatives (Peay et al. 2012). Priority effects can also increase phylogenetic diversity through facilitation when early-arriving species alters abiotic or biotic conditions favouring the establishment of distantly related species (Valiente-Banuet and Verdú 2007). For example, yeasts living in restrictive habitats like necrotic stems of columnar cactus facilitate the growth of distantly related species increasing thus the phylogenetic diversity of the yeast community (Anderson et al. 2004). In post-fire environments, EM fungal traits allowing a rapid colonization of the scarce roots such as the accumulation of abundant viable propagules in the soil, the speed of spore germination and hyphal growth, and the host compatibility can be decisive in defining priority effects (that can lead phylogenetic over-dispersion), and consequently in structuring EM communities (Kennedy et al. 2009).

On seedlings, the elapsed time since fire did not affect the overall phylogenetic diversity of the whole EM community, although this lack of effect could be the net result of some fungal clades being significantly overrepresented and others under-represented. The clades belonging to *Rhizopogonaceae* and *Atheliaceae* were clearly favoured in the site recently affected by fire, suggesting that fungi within each respective group could share specific traits of potential adaptation to fire. *Rhizopogon* spp. have been previously reported dominating EM communities associated with pine roots in post-fire settings (Horton et al. 1998; Baar et al. 1999; Rincón and Pueyo 2010; Buscardo et al. 2010), where some traits such as the production of abundant fruit-bodies and spores, the long-distance exploration type mycelium, the dispersal spore strategy by animals and the host preference towards *Pinus* spp. (Molina et al. 1999; Taylor and Bruns 1999; Agerer 2001; Bruns et al. 2002) could be decisive conferring competitive advantage to these species. Buscardo et al. (2010) showed that in maritime pine forest soils affected by fire, *Rhizopogon* spores accumulated, and even reported an alternate dominance of the species *R. luteolus* and *R. roseolus* depending on the fire return interval length. The clade corresponding to *Cortinariaceae*, with some species typically reported in mature pine forests, was significantly underrepresented at the most recently burnt site, an effect that could be related with traits such as the production of low amount of spores (Taylor and Bruns 1999; Nara 2009), or the preference of species within this group for organic forms of nitrogen (Lilleskov et al. 2002). Similarly, other fungi such as *Amanita phalloides* Scr., *Laccaria bicolor* (Maire) P.D. Orton and *Hebeloma cistophilum* Maire, were underrepresented in the site more recently affected by fire. Specifically, the two last fungus appeared on pine roots in the site affected by fire fourteen years ago probably indicating seedlings sharing diversity with other plants (i.e. *Cistus* spp. or pine saplings) via common myceliar networks.

Overall, our results indicate that the elapsed time after fire could act as a selective agent affecting the phylogenetic structure of the EM fungal communities of naturally regenerated young pines, pointing out to some groups as potentially fire-adapted fungi. Additional work is needed for better understanding the responses and underlying mechanisms governing the assemblage patterns of post-fire EM fungal communities and the meaning of their interaction with naturally regenerated plants, which is of prime importance for the recovery and conservation of Mediterranean forest ecosystems.

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References

Agerer R (1987–1998) Colour atlas of ectomycorrhizae. Einhirn-Verlag Eduard Dietenberger, Munich

Agerer R (2001) Exploration types of ectomycorrhizae. A proposal to classify extomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza 11:107–114

Anderson TM, Lachance M-A, Starmer WT (2004) The relationship of phylogeny to community structure: the cactus yeast community. Am Nat 164:709–721

Avis PG, McLaughlin DJ, Dentinger BC, Reich PB (2003) Long-term increase in nitrogen supply alters above and below-ground ectomycorrhizal communities and increases the dominance of *Russula* spp. in a temperate oak savannah. New Phytol 160:239–253

Baar J, Horton TR, Kretzer AM, Bruns TD (1999) Mycorrhizal colonization of *Pinus muricata* from resistant propagules after a stand-replacing wildfire. New Phytol 143:409–418

Bastias BA, Xu Z, Cairney JWG (2006) Influence of long-term repeated prescribed burning on mycelial communities of ectomycorrhizal fungi. New Phytol 172:149–158

Berbee ML, Taylor JW (1993) Dating the evolutionary radiations of the true fungi. Can J Bot 71:1114–1127

Branco S, Ree RH (2010) Serpentine soils do not limit mycorrhizal fungal diversity. PlosOne 5:1–7

Bruns T, Biartondo MI, Taylor L (2002) Host specificity in ectomycorrhizal communities: what do the exceptions tell us. Integr Comp Biol 42:352–359

Buscardo E, Rodríguez-Echeverría S, Martin MP, de Angelis P, Pereira JS, Freitas H (2010) Impact of wildfire return interval on the ectomycorrhizal resistant propagules communities of a Mediterranean open forest. Fungal Biol 114:628–636

Buscardo E, Freitas H, Pereira JS, de Angelis P (2011) Common environmental factors explain both ectomycorrhizal species diversity and pine regeneration variability in a post-fire Mediterranean forest. Mycorrhiza 21:549–558

Cairney JWG, Bastias BA (2007) Influences of fire on forest soil fungal communities. Can J For Res 37:207–215

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol 17:540–552

Certini G (2005) Effects of fire on properties of forest soils: a review. Oecologia 143:1–10

Claridge AW, Trappe JM, Mills DJ, Claridge DL (2009) Diversity and habitat relationships of hypogeous fungi. III. Factors influencing the occurrence of fire-adapted species. Mycol Res 113:792–801

Dahlberg A, Schimmel J, Taylor AFS, Johannesson H (2001) Post-fire legacy of ectomycorrhizal fungal communities in the Swedish boreal forest in relation to fire severity and logging intensity. Biol Conserv 100:151–161

Dickie IA, Dentinger BTM, Avis PG, McLaughlin DJ, Reich PB (2009) Ectomycorrhizal communities of oak savana are distinct from forest communities. Mycologia 101:473–483

Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol 7:214

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for Basidomycetes: application to identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Grogan P, Baar J, Bruns TD (2000) Below-ground ectomycorrhizal community structure in a recently burned bishop pine forest. J Ecol 88:1051–1062

Hart SC, DeLuca TH, Newman GS, MacKenzie MD, Boyle SI (2005) Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. For Ecol Manag 220:166–184

Hibbet DS (2006) A phylogenetic overview of the Agaricomycotina. Mycologia 96:917–925

Horner-Devine MC, Bohannan BJM (2006) Phylogenetic clustering and overdispersion in bacterial communities. Ecology S100-S108

Horton TR, Cazares E, Bruns TD (1998) Ectomycorrhizal, vesicular-arbuscular and dark septate fungal colonization of bishop pine (*Pinus muricata*) seedlings in the first 5 months of growth after wildfire. Mycorrhiza 8:11–18

James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J et al (2006) Reconstruction the early evolution of fungi using a six gene phylogeny. Nature 443:818–822

Jonsson L, Dahlberg A, Nilsson MC, Zackrisson O, Karen O (1999) Ectomycorrhizal fungal communities in late-successional Swedish boreal forests, and their composition following wildfire. Mol Ecol 8:205–215

Johnson D, Martin F, Cairney JWG, Anderson IC (2012) The importance of individuals: intraspecific diversity on mycorrhizal plants and fungi in ecosystems. New Phytol 194:614–628

Keeley JE, Bond WJ, Bradstock RA, Pausas JG, Rundel PW (2012) Fire in Mediterranean Ecosystems. Ecology, Evolution and Management. Cambridge University Press, NY

Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP, Webb CO (2010) Picante: R tools for integrating phylogenies and ecology. Bioinformatics 26:1463–1464

Kennedy PG, Peay KG, Bruns TD (2009) Root tip competition among ectomycorrhizal fungi: are priority effects a rule or an exception? Ecology 90:2098–2107

Kennedy P (2010) Ectomycorrhizal fungi and interspecific competition: species interactions, community structure, coexistence mechanisms and future research directions. New Phytol 187:895–910

Kipfer T, Moser B, Egli S, Wohlgemuth T, Ghazoul J (2011) Ectomycorrhizal succession patterns in *Pinus sylvestris* forests after stand-replacing fire in the Central Alps. Oecologia 167:219–228

LeDuc SD, Lilleskov EA, Horton TR, Rothstein DE (2012) Ectomycorrhizal fungal succession coincides with shifts in organic nitrogen availability and canopy closure in post-wildfire jack pine forests. Oecologia. doi:10.1007/s00442-012-2471-0

Legendre P, De Cáceres M, Bocard D (2010) Community surveys through space and time: testing the space-time interaction in the absence of replication. Ecology 91:262–272

Lilleskov EA, Hobbie EA, Fahey TJ (2002) Ectomycorrhizal fungal taxa differing in response to nitrogen deposition also differ in pure culture organic nitrogen use and natural abundance of nitrogen isotopes. New Phytol 154:219–231

Loepfe L, Martinez-Vilalta J, Oliveres J, Piñol J, Lloret F (2010) Feedbacks between fuel reduction and landscape homogenisation determine fire regimes in three Mediterranean areas. For Ecol Manag 259:2366–2374

Longo S, Urcelay C, Nouhra E (2011) Long term effects of fire on ectomycorrhizas and soil properties in *Nothofagus pumilio* foests in Argentina. For Ecol Manag 262:348–354

Magurran AE (1988) Ecological diversity and its measurement. Princeton University Press, USA, Princeton

Maherali H, Klironomos JN (2007) Influence of Phylogeny on Fungal Community Assembly and Ecosystem Functioning. Science 316:1746–1748

McMullan-Fisher SJM, May TW, Robinson RM, Bell TL, Lebel T, Catcheside P, York A (2011) Fungi and fire in Australian ecosystems: a review of current knowledge, management implications and future directions. Aust J Bot 59:70–90

Molina R, Trappe JM, Grubisha LC, Spatafora JW (1999) *Rhizopogon*. In: Cairney JWG, Chambers SM (eds) Ectomycorrhizal fungi key genera in profile. Springer, Berlin, Germany, pp 129–161

Moncalvo J-M, Nilsson RH, Koster B, Dunham SM, Bernauer T, Matheny PB, McLenon T, Margaritescu S, Wei BM, Garnica S et al (2006) The cantharelloid clade: dealing with incongruent gene trees and phylogenetic reconstruction methods. Mycologia 98:937–948

Nara K (2009) Spores of ectomycorrhizal fungi: ecological strategies for germination and dormancy. New Phytol 181:245–248

Nilsson RH, Kristiansson E, Ryberg M, Hallenberg N, Larsson KH (2008) Intraspecific ITS variability in the Kingdom Fungi as expressed in the international sequence databases and its implications for molecular species identification. Evol Informat 4:193–201

Nilsson LO, Wallander H, Gundersen P (2012) Changes in microbial activities and biomasses over a forest floor gradient in C to N ratio. Plant Soil 355:75–86

Oksanen JF, Blanchet G, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H (2011) Vegan: Community Ecology Package. R package version 2.0-0. http://CRAN.R-project.org/package=vegan

Pausas JG, Verdú M (2010) The jungle of methods for evaluating phenotypic and phylogenetic structure of communities. BioScience 60:614–625

Peay KG, Kennedy PG, Davies SJ, Tan S, Bruns TD (2010) Potential link between plant and fungal distributions in a dipterocarp rainforest: community and phylogenetic structure of tropical ectomycorrhizal fungi across a plant and soil ecotone. New Phytol 185:529–542

Peay KG, Belisle M, Fukami T (2012) Phylogenetic relatedness predicts priority effects in nectar yeast communities. P Roy Soc B 279:749–758

Pereira P, Úbeda X, Martin DA (2012) Fire severity effects on ash chemical composition and water extractable elements. Geoderma 191:105–114

R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.rproject.org/

Rincón AR, Pueyo JJ (2010) Effect of fire severity and site slope on diversity and structure of the ectomycorrhizal fungal community associated with post-fire regenerated *Pinus pinaster* Ait. seedlings. For Ecol Manag 260:361–369

Rodrigo A, Retana J, Pico FX (2004) Direct regeneration is not the only response of Mediterranean forests to large fires. Ecology 85:716–729

Smith SE, Read DJ (1997) Mycorrhizal Symbiosis. Academic Press, Cambridge, UK

Stendell ER, Horton TR, Bruns TD (1999) Early effects of prescribed fire on the structure of the ectomycorrhizal fungus community in a Sierra Nevada ponderosa pine forest. Mycol Res 103:1353–1359

Schoch CL, Sung GH, Pez-Giraldez FL, Townsend JP, Miadlikowska J, Hofstetter V, Robertse B, Matheny PB, Kauff F, Wang Z et al (2009) The Ascomycota tree of life: a phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. Systematic Biol 58:224–239

Sugiyama J, Hosaka K, Suth SO (2006) Early diverging Ascomycota: phylogenetic divergence and related evolutionary enigmas. Mycologia 98:996–1005

Tapias R, Climent J, Pardos JA, Gil L (2004) Life history of Mediterranean pines. Plant Ecol 171:53–68

Taylor DL, Bruns TD (1999) Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: minimal overlap between the mature forest and resistant propagule communities. Mol Ecol 8:1837–1850

Tedersoo L, Suvi T, Larsson E, Koljalg U (2006) Diversity and community structure of ectomycorrhizal fungi in a wooded meadow. Mycol Res 110:734–748

Thompson M, Calkin DE (2011) Uncertainty and risk in wild land fire management: a review. J Environ Manage 92:1895–0909

Torres P, Honrubia M (1997) Changes and effects of a natural fire on ectomycorrhizal inoculum potential of soil in a *Pinus halepensis* forest. For Ecol Manag 96:189–196

Treseder KK, Mack MC, Cross A (2004) Relationships among fires, fungi, and soil dynamics in Alaskan boreal forests. Ecol Appl 14:1824–1838

Tweig BD, Durall DM, Simard SW (2009) Influence of soil nutrients on ectomycorrhizal communities in a chronosequence of mixed temperate forests. Mycorrhiza 19:305–316

Valiente-Banuet A, Verdú M (2007) Facilitation can increase the phylogenetic diversity of plant communities. Ecol Lett 10:1029–1036

Webb CO, Ackerly DD, McPeek MA, Donoghue MJ (2002) Phylogenies and community ecology. Annu Rev Ecol Syst 33:475–505

Webb CO, Ackerly DD, Kembel SW (2008) Phylocom: software for the analysis of phylogenetic community structure and trait evolution. Bioinformatics 24:2098–2100

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR Protocols—A Guide to Methods and Applications. Academic Press, New York, pp 315–322