Abstract: Most oak woodlands in the Mediterranean basin have been traditionally converted into agro-silvo-pastoral systems called montado that combined both a successful biological conservation and soil productivity. However, in Portugal, in line with the trend of other European countries, profound changes in management options during the 20th century have lead to landscape simplification, through both intensification of land use practices and abandonment. Ectomycorrhizae (ECM) are recognized as key components of soil biological processes in mixed forests, including drought tolerance. In this work, we examined the influence of land use activities on ECM fungi richness and abundance in the montado ecosystem. Mycorrhizal symbiosis was investigated during the summer time allowing for ECM communities functioning below-ground during a critical period under Mediterranean climate influence. The study was conducted in 15
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In total 55 active ECM taxa were recorded in the 15 montado areas. ECM were identified through their morphological and anatomical characteristics and by nrDNA internal transcribed spacer (ITS) sequences. The species Cenococcum geophilum and the families Russulaceae and Thelephoraceae explained 56.4% of ECM community structure during the drought period. Cenococcum geophilum was the only active ECM species common to all stands. Multivariate analysis revealed that ECM species richness was positively correlated with low mortality of cork oak, while ECM relative abundance was positively correlated with crop rotation cultures and soil tillage. Land use management planning effects on ECM fungi community and its implications for oak woodlands sustainability in scenarios of landscape simplification, cork oak mortality and global warming are discussed.
Title:

Influence of land use practices in ectomycorrhizal fungi richness and diversity in managed oak woodlands under semi-arid Mediterranean climate conditions

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Abstract

Most oak woodlands in the Mediterranean basin have been traditionally converted into agro-silvo-pastoral systems called montado that combined both a successful biological conservation and soil productivity. However, in Portugal, in line with the trend of other European countries, profound changes in management options during the 20th century have lead to landscape simplification, through both intensification of land use practices and abandonment. Ectomycorrhizae (ECM) are recognized as key components of soil biological processes in mixed forests, including drought tolerance. In this work, we examined the influence of land use activities on ECM fungi richness and abundance in the montado ecosystem. Mycorrhizal symbiosis was investigated during the summer time allowing for ECM communities functioning below-ground during a critical period under Mediterranean climate influence. The study was conducted in 15 stands established in nine typical montados with agro-silvo-pastoral exploitation, located in southern Portugal (Alentejo). Land use history over the last 25 years, tree vitality, climatic and edaphic conditions were considered.

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Key words: ectomycorrhizal fungi, land use, soil diversity, Quercus suber, Mediterranean ecosystems

Introduction

Management of agro-ecosystems and forestry in order to sustain productivity and to preserve biodiversity has become a major challenge in the last decades (Decocq et al., 2004). In large areas of the southern Iberian Peninsula (ca. 6.5 million ha; DGF, 2003), traditional land use systems correspond to managed oak woodlands (montado in Portugal). These ecosystems consist of open oak formations (usually Quercus suber L., Q.
rotundifolia L. and *Q. ilex* L.) with scattered tree cover (60–100 trees per ha) and one or two vegetation strata at the ground level, composed by shrubs and/or herbaceous plants (pastures and agricultural fields, in a 9-years rotation culture system (Pinto-Correia, 1993). Traditionally, managed ecosystems and the use of their multiple products and services (e.g., cork, wood, meat, fruits, apiculture, medicinal plants, mushrooms, habitat for fauna, tourism) have accompanied human history in the area and represent a classic example of sustainable land use in Europe (Joffre et al., 1999; Pinto-Correia, 1993; Pulido et al., 2001; Pinto-Correia and Vos, 2004), by combining biological conservation and socio-economical value.

Despite of the ecophysiological adaptations of oak woodlands to climate environment (Nardini et al., 1999; Chaves et al., 2002; Kurz-Besson et al., 2006), successive disturbances over the second half of 20th century have dramatically changed the landscape in Portuguese oak woodlands. This resulted from rapid technological and economic changes, increased opening of markets, and common European agricultural policies. Management of oak woodlands has being based on the intensification of the land use practices, with frequent crop rotation cultures, high inputs of fertilizers, pesticides and herbicides, and forestation with eucalypt trees (*Eucalyptus globulus* Labill.). Additionally, large areas of oak woodlands have being abandoned and underwent the natural process of ecological succession (Pinto-Correia, 1993; Debussche et al., 1999; Pinto-Correia and Vos, 2004).

Through this process, rural areas were progressively re-colonized by autochthonous vegetation, changing from herbaceous dominancy or silvopastoral systems with shrubs in low density, to shrub-dominated areas. Such reestablishment of shrubs increases the risk of fire (Nunes et al., 2005) and plant diseases (Brasier, 1996), which lead to over-exploitation of soil resources (Pinto-Correia and Mascaranhas, 1999) and affect negatively plant community resilience (Díaz-Delgado et al., 2002). In southern Portugal, the decline of managed oak woodlands has further driven the loss of habitats (Pinto-Correia and Mascaranhas, 1999) and the loss of microbial, plant and animal diversity and productivity (Hector et al., 1999; Da Silva et al., 2008). The situation is extended to most Mediterranean landscapes of southern Europe (Díaz-Delgado et al., 2002; Mouillot et al., 2005; Rios-Díaz et al., 2006). The increasing evidence of heavy mortality and decline of evergreen oaks (*Quercus suber* and *Q. ilex* subsp. *rotundifolia*), but also the greatest warming in Europe over the last decades (European Environment Agency, 2004), highlights the necessity of understanding the effects of the current land use practices on the productivity and conservation of soil microorganisms. Among the symbiotic soil microorganisms, there is scarce information about the role of ectomycorrhizae (ECM) functioning in biogeochemical cycles, soil
microbial ecology and in plant community structure in oak woodlands, classified as highly susceptible to
degradation.
Recent studies have shown that traditional land use systems cause lower levels of disturbance and enhance the
biodiversity of flora and fauna rather than reduce it, thus agro-silvo-pastoral landscapes can support a greater
overall biodiversity than climax European woodlands from which the first have derived (Decocq et al., 2004;
Pinto-Correia and Vos, 2004; Rois-Díaz et al., 2006). ECM fungi are widely recognized for their benefits to
plants, including drought tolerance, pathogen resistance and protection against toxic compounds (see Smith and
Read, 2008). The mycorrhizae mycelium network plays a vital role in plant nutrition (Perez-Moreno and Read,
2000) as well as in nutrient cycling (Högberg and Högberg, 2002; Read and Perez-Moreno, 2003; Leak et al.,
2004), influencing seedling establishment, plant diversity, and vegetation community dynamics. However, little
is known about the effects of land use practices on ECM fungi richness and abundance below-ground in oak
woodlands. We have compiled information on land use history over the 25-years prior to this study and
combined qualitative data related to management planning strategies with ECM fungi richness and diversity
patterns on cork oak roots. The main objectives of the present study were: (1) to determine ECM fungi richness
and abundance in Portuguese managed oak woodlands during summer time, a critical period under
Mediterranean climate influence, (2) to discuss the relationships between ECM fungi community and land use
practices, (3) to bring new insights into the role of ECM fungi in managed woodlands under drought conditions
towards cork oak vitality and ecosystem sustainability.

Materials and Methods

Study site and experimental design
This study was conducted in fifteen stands of 500 m² selected in nine oak woodlands (montado) from southern
Portugal (Alentejo region) (Table 1), characterized by open cork oak formations under different land use
management planning. Areas with the best cork quality were selected taking into account the commitment of
land users and stakeholders. Two stands (a and b) were selected per montado. The two stands from the same
montado have the same management plan regime, but may comprise differences in land use practices,
vegetation cover composition and cork oak mortality (Table 2). Two montado ecosystems have only one stand
selected, DA-montado corresponding to the abandonment situation, and SB-montado the smallest managed oak
woodland. The land use history over the previous 25 years was obtained by interviewing farmers, land users and

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stakeholders and is summarized in Table 2. The following parameters were assessed: cutting practices, methods and frequency; crop rotation, including the introduction of fertilizers versus rotation with legume crops; intensity of silvo-pastoral exploitation; vegetation cover and density of dominant species; cork oak mortality; cork exploitation (over-harvesting) and cork quality and intensity of mushroom collection. Vegetation surveys were conducted considering the dominant species at the peak of vegetation cover, from May to July 2000 (Table 1). Sampling of ECM was performed during summer, between June and August 2000. Rhizospheric soils were sampled and K, Na, Ca, Mg were determined after an ammonium acetate extraction at pH 7 (Chapman, 1979). Soil organic matter was determined by the Tinsley method (L.Q.A.R.S., 1977); pH was measured by a glass electrode on 2.5:1 distilled water: soil suspension (L.Q.A.R.S., 1977).

**Sampling of ectomycorrhizae**

Four cork oak trees were selected per study stand. For each tree, four 10x10x15 cm (= 1500 cm³) monoliths of soil were collected along the four main cardinal directions (N, E, S, W). Rhizospheric soil was taken at a 60 cm distance from the trunk to ensure that the ECM fungi belonged to cork oak tree. The four monoliths per tree were wrapped separately in wet newspaper separately and sealed in plastic bags. In the laboratory, the four monoliths per tree were pooled and one 1500 cm³ sample of crumbled soil with roots was considered for ECM assessment. These subsamples were stored at 4°C for up to 2 weeks until they were processed. Soil particles adhering to roots were removed in water with pipettes, fine forceps and preparation needles, using a Wild Heerbrugg stereomicroscope. A total of 260 545 cork oak root tips (RTip) were analysed, and sorted into mycorrhized (ECM) and non-mycorrhized tips (nECM) by the presence of mantle and emanating elements. Mycorrhized tips were then separated into active and inactive tips (aECM and iECM, respectively); the ECM tips with wrinkled mantle were considered as inactive tips. The active ECM roots were categorized into morphotypes using a Wild Heerbrugg stereomicroscope. Morphotypes were assigned according to the morphology of the mantle and its emanating elements, i.e., cystidia, laticiferous hyphae, emanating hyphae and rhizomorphs, and further specified regarding their microscopical features into anatomotypes (Agerer, 1987-2008; Agerer and Rambold, 2004-2007). Further classification of the fungi was done through direct DNA sequencing. Detailed descriptions of all aECM, photos, drawings and slides, are available from the corresponding author and have been already published for most of the types (see Appendix). Voucher specimens were preserved in alcohol 50% and in 2% CTAB, and are kept in the Department of Botany of the University of
Coimbra (COI).

**DNA analysis**

Fungal DNA MiniPrep Kit (Omega, Biotech, Doraville, USA) was applied and ITS nrDNA amplified by nested PCR using the primer pair ITS1F/ITS4B (Gardes and Bruns, 1993) followed by ITS1/ITS4 (White et al., 1990). PCR reactions were done preparing individual reactions to a final volume of 25 μl with Ready-To-Go PCR Beads (Amersham-Pharmacia Biotech) as mentioned in Winka et al. (1998) with a final DNA concentration around 0.25 ng/μl. The PCR product was purified using QIAquick Gel PCR purification kit (QIAGEN Inc., Chatsworth, CA, USA) according to the manufacturer’s instructions. Fragments were sequenced using the same primers as mentioned above at the DNA Automatic Sequencing Service (SSAD, CIB-CSIC, Madrid, Spain). Sequencer (Gene Codes Corporation, Ann Arbor, Michigan, USA) was used to recognize the consensus sequence from the two strands of the ITS nrDNA. The new sequences have been stored in the EMBL database (see Appendix). A nucleotide-nucleotide search (Blast) was done at the National Center of Biological Information (NCBI) using BLAST (Altschul et al., 1997).

**Data analysis**

ECM fungi community diversity was described by using the parameters RTip (root tips), ECM (mycorrhized tips), nECM (no-mycorrhized tips), aECM (active mycorrhized tips) and iECM (inactive mycorrhized tips). The RTip was assessed by the cumulative number of the total root tips, i.e., RTip = ∑ aECM + ∑ iECM + ∑ nECM (Azul, 2002). Within the mycorrhizal root tips, the relative abundance of a given morphotype (X_ECM) was defined as the cumulative number of X active ectomycorrhizae per aECM (Azul, 2002).

A nested ANOVA (Zar, 1986) was used to compare all above parameters between the 15 stands and between each montado. Normality and homoscedasticity were tested by the Kolmogorov-Smirnof and Bartlett tests, respectively. Mean differences were compared with Tukey’s test at 5% level of significance. When either one or both of these assumptions were not met the data were transformed following Zar’s (1986) recommendations. ECM diversity at a given study stand was estimated by the following descriptors: i) species richness, i.e., number of ECM species found per plot (S); ii) Shannon-Wiener (H) and Simpson (λ) diversity indexes; iii) Pielou evenness (H'); iv) Margalef (D), Log a (S) and Jack-Knife richness indexes; and v) Whittaker β-diversity index (Magurran, 1988). The relationship between ECM diversity descriptors and land use variables (coded...
from Table I) was done using a Redundancy Analysis (RDA). A forward selection procedure was performed to determine those explanatory land use variables with a significant relationship with the ectomycorrhizae data. Monte-Carlo permutation tests were used to assess the significance of these relationships and also the significance of the obtained canonical axes. Univariate statistical analyses were performed using STATISTICA 6.0 software package (StatSoft, 2001) and multivariate analyses were done using the CANOCO 4.5 software (Ter Braak and Smilauer, 2002). Data analysis was applied to the morphotypes more abundant in cork root samples; only active ectomycorrhizae (aECM) with more than 5% relative abundance were considered.

**Results**

**Ectomycorrhizal fungi richness in managed oak woodlands**

In total, 55 ECM taxa were identified in the 15 stands based on morphological and anatomical features or determined by DNA sequencing (see Appendix). Figure 1 shows the relative abundance of the most representative active ECM (aECM) genera and families in the managed oak woodlands under drought conditions. The species *Cenococcum geophilum* and the families *Russulaceae* and *Thelephoraceae* represented altogether 56.4% of ECM taxa in the 15 stands. The genera *Russula* and *Tomentella* were the most rich in species, with 13 and 15 taxa respectively (see Appendix). The families *Cortinariaceae* and *Boletaceae* were represented with five and two taxa, respectively; the genera *Lactarius* and *Amanita* and were represented with four and two taxa, respectively. The genera *Genea*, *Pisolithus*, *Scleroderma*, and *Tuber* were the fewest represented, with just one ECM taxa present (see Appendix). *Cenococcum geophilum* was the only ECM species common to all stands (Fig. 1).

ECM communities below-ground exhibited high heterogeneity in species composition in the 15 stands (Fig. 1). Such heterogeneity was also reflected in the ECM richness and abundance values (Table 3). A maximum of 20 taxa was observed in the FM-*montado* with extensive silvopastoral exploitation and shrubs artificially maintained at 25-35% of density cover (Table 3). SB-*montado* and AM-*montado*, with 17 and 15 taxa respectively, are currently under silvopastoral exploitation with shrub controlled by cattle (present sporadically). The lowest values, both with 9 taxa, were observed in the ES-*montado*, with crop rotation cultures with legumes plus nutrient supply (Crop Rot_L&N) and in the DA-*montado* with no land use management over the last 15-years (with shrubs occupying 95% of vegetation cover) (Tables 2 and 3). The variation pattern observed in the cumulative number of taxa between the nine *montados* is reflected in the species richness (Margalef) values,
with a clear decrease in ES-montado and DA-montado but also in the LT-montado, with mixed cork oak formations with pine (*Pinus pinaster*). Significant differences were obtained for the average of aECM taxa \(F_{0.05(1),6}=11.4; \ p<0.01\) among the nine montados (Fig. 2), i.e. considering the management planning strategy in the oak woodlands. The aECM abundance was not correlated with ECM richness \(r=-0.33; \ p>0.05\). The aECM abundance showed to be a parameter more changeable. Significant changes were observed in the average aECM relative abundance between the 15 stands \(F_{0.05(1),6}=5.45; \ p<0.05\; \text{Fig. 4}\). Highest values of total aECM abundance were observed in the stand b of OG-montado with extensive silvopastoral exploitation of sheep, in the two stands of VC-montado (recent shrub cutting with soil tillage) and in the stand b of ES-montado (under crop rotation cultures) (Fig. 4). No significant differences between montados were found in the number of mycorrhizal (ECM) and non-mycorrhizal (nECM) \(F_{0.05(1),6}=3.93; \ p>0.05\, \text{for ECM and } F_{0.05(1),6}=2.05; \ p>0.05, \text{for nECM}\). Within the ECM, also no significant differences between montados were found between aECM and iECM \(F_{0.05(1),6}=1.05; \ p>0.05\, \text{for aECM and } F_{0.05(1),6}=1.80; \ p>0.05, \text{for iECM}\). However a high variability on both parameters was found within each montado \(F_{0.05(1),6}=4.01; \ p<0.01\, \text{for aECM and } F_{0.05(1),6}=3.78; \ p<0.01, \text{for iECM}\) (Table. 3). Regarding the total root tips (RTip), significant differences were found \(F_{0.05(1),6}=5.45; \ p<0.05\; \text{Fig. 3}\) among the nine montado areas. Highest values of total RTip were registered in the OG-montado and in the ES-montado.

**Relationships between montado descriptors and ECM fungi diversity**

The results from the redundancy analysis showed that ECM fungi diversity descriptors were significantly related to some environmental variables. After the forward selection procedure six variables were selected (all with \(p<0.05\) after the Monte-Carlo test) considering their influence in the ECM fungi diversity descriptors: land use practices with crop rotation cultures using legumes and nutrient supply, \(\text{CropRot}_L&N\), recent soil tillage \(\text{Mobil}_0\), oak woodlands mixed with pine (pine), low mortality of cork oak \(\text{Low}_M\), sodium content in soil \(\text{Na}\) and mean annual temperature \(\text{Av}_\text{temperature}\) (Fig. 5). These six variables explained 80% of the variation of the ECM fungi diversity data, with axis 1 and axis 2 representing 42.9% and 28.1% of that variability, respectively. Monte-Carlo test revealed a significant relationship between these environmental variables and the ECM fungi diversity descriptors \(F=4.181, \ p<0.01\ \text{for the first axis; } F=5.348, \ p<0.01\ \text{considering all axes together}\).
There was a variation among *montados* sites in soil chemistry (Table 4). However, only Na showed to be a significant variable in the forward selection procedure of the RDA. The lowest values of pH were noticed in the *montados* LTA and LTB with cork oak mixed with pine. The lowest values of K were obtained in the *montado* AMb with no cork mortality and in the *montados* CÇ, ESA and ESb with 2-2.5 dead trees/ha. The variables Ca and Mg exhibited higher heterogeneity among the stands.

Cork oak mortality (Low_Mort) was one of the key features affecting the ECM fungi richness below-ground (Shannon index) (Fig. 5). The *montados* presenting an absent (FM, AM) or a low (SB, OG, VC) cork oak mortality exhibited higher ECM fungi richness and diversity (Tables 2 and 3).

ECM richness was greatest in FM-*montado* (Table 3) with no cork oak mortality and where shrubs are kept at up 35% of total vegetation cover density. In spite of this, no significant relationship between ECM richness and diversity were obtained in relation to the shrub strata (the presence of shrubs was not a significant variable in the forward selection procedure of the RDA).

The land use practices affecting more negatively ECM fungi richness below-ground were the crop rotation cultures with legumes and nutrient supply (Crop Rot_L&N), and the recent soil tillage (Mobil_0) (Fig. 5). ECM richness and diversity were also negatively correlated with cork oak woodlands mixed with pine (*Pinus pinaster* and/or *P. pinea*) (Fig. 5).

The ECM fungi richness and abundance were both influenced by the land use practices (Crop Rot_L&N; Mobil_0) and geographical localization (here represented by the mean annual temperature – Av_Temp) (Fig. 5). Regarding the ECM abundance, positive correlations were observed with the crop rotation cultures, with legumes and nutrient supply (Crop Rot_L&N), and also with the recent soil tillage (Mobil_0) (Fig. 5).

**Discussion**

The present study confirms that land use practices conducted in managed oak woodlands influence ECM fungi richness and abundance below-ground. It was possible to establish a trend using ECM fungi diversity descriptors and land use history, tree vitality, climatic and edaphic conditions. The ECM diversity was higher in oak woodlands with low cork oak mortality and under extensive silvo-pastoral exploitation where shrub strata are artificially maintained at low densities. Altogether, 55 taxa of aECM were identified in the 15 stands, demonstrating that ECM community functioning is quite diverse in structure even during drought. The ubiquitous species *Cenococcum geophilum* and the members of families *Russulaceae* and *Thelephoraceae*...
represented 56.4% of the aECM taxa identified. Similar tendency for the dominance of *C. geophilum* and the families *Russulaceae* and *Thelephoraceae* in ECM communities has been showed during spring time in an old-growth *Quercus ilex* forest in Corsica (Richard et al., 2005) and in forests from northern hemisphere (Gardes and Bruns 1996; Horton and Bruns 1998; Tedersoo et al. 2003; Lilleskov et al. 2004; Baier et al. 2006). *C. geophilum* and thelephoroid ECM were among the most frequent taxa observed over a 3-year period in a managed *Q. ilex* forest from northern Iberian Peninsula (De Román and De Miguel, 2005). Earlier studies conducted in managed *Q. suber* woodlands showed that ECM fungi community exhibited high diversity in species composition (Azul, 2002; Azul et al., 2006 a-h; Azul et al., 2008 a-e), but was clearly influenced by the seasonality effect. Azul (2002) reported an apparent renovation in ECM community composition particularly between spring and summer, with an increase in members of *Russulaceae* and *Thelephoraceae* during summer time; the lowest values of ECM richness and abundance were recorded in autumn. Seasonal fluctuations were also reported in managed *Q. ilex* forest, with a significant decrease in ECM richness during summer (De Román and De Miguel, 2005). Further studies in ECM community functioning during summer time are desirable in order to better understand which factors may influence ECM richness and abundance below-ground under drought conditions.

Low cork oak mortality was a central factor affecting the ECM fungi richness and diversity below-ground in managed oak woodlands, suggesting that ECM were sensitive indicators of cork oak vitality. The aECM species richness peaked in the stand b of FM-montado and in the two stands of OG-montado, both with low (or absent) cork oak mortality. The overall ECM diversity seemed to be closely related to the management planning strategy and the highest in ECM taxa were observed in the stands with low cork oak mortality and where shrubs are artificially maintained at low. This trend is of high importance since sustainability is strongly dependent on abiotic conditions and on biotic interactions that occur prior to and after disturbance (Decocq et al., 2004). In the present study, we did not test whether the shrubs contributed to ECM fungi diversity below-ground by establishing mycelial networks with *Q. suber*. However, it is known that in ecosystems where multi-host ECM fungi dominates, the percentage of host taxa shared can be high (Horton and Bruns, 1998; Kennedy et al., 2003). The maintenance of diversity in mycorrhizal communities may be regarded as a strategy to ensure nutrient cycling, important factor for the vegetation dynamics and oak vitality. The abandonment of land use practices in oak woodlands seemed to lead to simplification of ECM community below-ground. The lowest values of ECM fungi richness and diversity were observed in DA-montado, abandoned 15 years ago and with shrubs 2 m high.
occupying ca. 95% of total vegetation cover. The increase of shrub density is of great significance for
agroforestry management since shrubs are the most ignitable cover type when compared with mosaics of diverse
agriculture and annual crops (Nunes et al., 2005). Such increasing risk of fire can lead to long-term negative
effects on vegetation resilience (Diaz-Delgado et al., 2002) and nutrient cycling (Carreira et al., 1997). Our
findings sustain that shrub management, such as the frequency of cutting practices and the introduction of heavy
machinery, involving or not soil tillage, may affect ECM diversity below-ground in managed oak woodlands.
Thus, it is important to study ECM functioning during summer and analyse their importance for oak vitality and
sustainability. The effects of agroforestry may be reflected on ECM fungi species richness and evenness
(Hagerman et al., 1999; Avis et al., 2003), and they are directly correlated with ecological mechanisms by which
plant variability, productivity and biodiversity are re-established (Simard and Durall, 2004). Hagerman and
Durall (2004) reported that the conservation of refuge species following clear cutting increased the opportunity
of out-planted seedlings to become colonized by a more diverse ECM fungal community. The preservation of
patches of autochthonous vegetation in oak woodlands play a crucial role, also in the conservation of soil
macrofauna (Sousa et al., 2004; Da Silva et al., 2008), in the establishment of rare plants species (Lavergne et
al., 2005), and in the protection of small mammals and birds that are more dependent on specific habitats (Stoatc
et al., 2000; Klaa et al., 2005).
The traditional rotation cultures in managed oak woodlands comprises periods with low vegetation cover at
ground level due to soil tillage, crop production and shrub cutting, which reduce the nutrient uptake and
contribute for losses due to leaching (Pinto-Correia and Vos, 2004). Management planning combining one-two
strata of vegetation at the ground level diminishes light as a limiting factor (Moreno et al., 2005), which is
considered one of the factors determining vegetation cover diversity in silvicultural ecosystems (Decoq et al.,
2004). In the present study, we observed that the total number of root tips peaked both stands of the ES-\textit{montado}
(crop rotation cultures with legumes and nutrient supply) and in the VC-\textit{montado} (recent shrub cutting with soil
tillage). Our findings sustain the hypothesis that either the introduction or the maintenance of one-two
vegetation strata, with shrubs at low densities during fallows, contributes to ECM fungi renovation below-
ground. This niche separation may favour the extension of the fine roots, increasing the number of root tips
susceptible to be colonized by ECM fungi (see Fig. 5). For the ecosystem productivity, such rooting pattern
ensures the rapid turnover of the fine root system, which represents a crucial pathway of carbon and nutrient
flow from plant to soil (Baddeley and Watson, 2005).
The assumption of establishing a compromise between management planning allowing for the conservation of ECM fungi diversity and abundance below-ground is urgent in the context of oak decline. Our results highlight that are land use practices that preserve more the ECM fungi richness and diversity below-ground than others, and also that the ECM richness and diversity may be closely related to low cork oak mortality. It is imperative to identify strategies that contribute to the increase of entire ecosystem resilience. Oak woodlands are extremely susceptible to degradation and there are factors more difficult to control such as the effects of global warming. Iberian Peninsula registered the greatest warming in Europe, and is already affected by an increasing trend in temperature and a decreasing precipitation (European Environment Agency, 2004). These climatic changes and climatic instabilities may favor the establishment and the aggressiveness of forest pathogens such as *P. cinnamomii*, strongly implied in cork oak mortality (Brasier 1996; 2000). Additional studies in summer time in managed oak woodlands are needed to afford biological and ecological information to better understand the role of the ECM fungi as biological mediators to prevent cork oak mortality, but also the productivity and sustainability.

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Trees in a Savannah-Type Mediterranean Ecosystem and its Contribution to the Local

influencing rare plant local occurrence, extinction and persistence: a 115-year study in the

and influence: the role of mycorrhizal mycelium in controlling plant communities and

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Table 1. Localization of the managed oak woodlands (typical *montado*) in study and characterization of its vegetation cover. The *montado* areas were selected along the main cork production area.

<table>
<thead>
<tr>
<th>Study site</th>
<th>Location</th>
<th>Vegetation cover</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ponte Sor (LT)</td>
<td>39°08'02&quot;N,</td>
<td>LTa and LTb - <em>Q. suber</em> (canopy 30 %) + <em>Pinus pinaster</em> Aiton (canopy 20 %);</td>
</tr>
<tr>
<td></td>
<td>8°12'36&quot;W</td>
<td>shrubs 25% density, mainly <em>Cistus</em> sp.</td>
</tr>
<tr>
<td>Coruche (CÇ)</td>
<td>38°56'25&quot;N,</td>
<td>CÇ - <em>Q. suber</em> (canopy 60-80 %), shrubs 50 % density,</td>
</tr>
<tr>
<td></td>
<td>8°18'11&quot;W</td>
<td>mainly <em>Cistus</em> sp.</td>
</tr>
<tr>
<td>Montemor-o-Novo (FM)</td>
<td>38°41'10&quot;N,</td>
<td>FMa and FMb - <em>Q. suber</em> (canopy 50 %); shrubs 20-30 % density, mainly <em>Cistus</em> sp.</td>
</tr>
<tr>
<td></td>
<td>8°20'23&quot;W</td>
<td></td>
</tr>
<tr>
<td>Alcácere do Sal (AM)</td>
<td>38°32'02&quot;N,</td>
<td>AMa - <em>Q suber</em> (canopy 70 %), shrubs 5-10 % canopy,</td>
</tr>
<tr>
<td></td>
<td>8°23'17&quot;W</td>
<td>mainly <em>Cistus</em> sp. AMb - <em>Q. suber</em> (canopy 80 %);shrubs 90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>% density (<em>Calluna vulgaris, Cistus ladanifer, Genista triacantha, Lavandula sp. and Ulex sp.</em>)</td>
</tr>
<tr>
<td>Alcácere do Sal (SB)</td>
<td>38°32'02&quot;N,</td>
<td>SB - <em>Q. suber</em> (canopy 30 %), <em>Q. rotundifolia</em> (canopy 20 %) and <em>Pinus pinea</em> (canopy 10 %); shrubs 5-10 % density (Cistus sp., Genista triacantha, Lavandula sp.)</td>
</tr>
<tr>
<td></td>
<td>8°23'17&quot;W</td>
<td></td>
</tr>
<tr>
<td>Grândola (ES)</td>
<td>38°11'45&quot;N,</td>
<td>ESa and ESb - <em>Q. suber</em> (canopy 30 %) + <em>Pinus pinea</em> (canopy 20 %); shrubs 5-10 % density, (Cistus sp., Genista triacantha, Chamespartum tridentatum and Ulex sp.)</td>
</tr>
<tr>
<td></td>
<td>8°29'13&quot;W</td>
<td></td>
</tr>
<tr>
<td>Santiago do Cacém (OG)</td>
<td>38°13'24&quot;N,</td>
<td>OGa - <em>Q suber</em> (canopy 20 %), <em>Pinus pinea</em> (canopy 10 %) and <em>Pyrus bourgaena</em> (vestigial); shrubs 20-30 % density (Arbutus unedo, Cistus ladanifer, C. populifolius, C. salvifolius, Genista triacantha), OGb - <em>Q. suber</em> (canopy 80 %), shrubs 60-70 % vegetation cover (Cistus crispus, C. ladanifer, C. salvifolius, Lavandula pedunculata, Lavandula sp., Genista triacantha and Ulex sp.)</td>
</tr>
<tr>
<td></td>
<td>8°28'19&quot;W</td>
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</tr>
<tr>
<td>Santiago do Cacém (DA)</td>
<td>38°13'32&quot;N,</td>
<td>DA - <em>Q. suber</em> (canopy 50 %), shrubs 95 % density, mainly <em>Cistus</em> sp.</td>
</tr>
<tr>
<td></td>
<td>8°29'03&quot;W</td>
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</tr>
<tr>
<td>Grândola (VC)</td>
<td>38°03'37&quot;N,</td>
<td>VCa and VCb - <em>Q. suber</em> (canopy 20 %)</td>
</tr>
<tr>
<td></td>
<td>8°33'41&quot;W</td>
<td></td>
</tr>
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</table>
Table 2. Environmental variables related to the land use history over the last 25-years in all studied *montado* areas. The cork is the main product in all *montado* areas and it is extracted each 9-10 years (*Shrub cutting done manually before the period under consideration).

<table>
<thead>
<tr>
<th>Environmental variables</th>
<th>LTa</th>
<th>LTb</th>
<th>CÇb</th>
<th>FMa</th>
<th>FMb</th>
<th>AMa</th>
<th>AMb</th>
<th>SbA</th>
<th>ESa</th>
<th>ESb</th>
<th>OGa</th>
<th>OGb</th>
<th>DAa</th>
<th>VCa</th>
<th>VCb</th>
</tr>
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<tbody>
<tr>
<td>Cork oak canopy: trees/ha</td>
<td>45</td>
<td>10</td>
<td>40</td>
<td>53</td>
<td>53</td>
<td>30</td>
<td>60</td>
<td>38</td>
<td>30</td>
<td>15</td>
<td>60</td>
<td>60</td>
<td>70</td>
<td>60</td>
<td>60</td>
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<tr>
<td>Cork oak mortality: trees/ha</td>
<td>1.3</td>
<td>2</td>
<td>1.2</td>
<td>0.3</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
<td>2.5</td>
<td>2.5</td>
<td>0.2</td>
<td>0</td>
<td>1.5</td>
<td>0.5</td>
<td>0.5</td>
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<tr>
<td>Shrub density cover (%)</td>
<td>≤ 35</td>
<td>≤ 35</td>
<td>± 50</td>
<td>≥ 35</td>
<td>≤ 35</td>
<td>≥ 90</td>
<td>± 50</td>
<td>± 50</td>
<td>± 50</td>
<td>≥ 90</td>
<td>≤ 35</td>
<td>≤ 35</td>
<td>± 50</td>
<td>± 50</td>
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<tr>
<td>Open oak formation</td>
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<tr>
<td>Cork oak with pine</td>
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<tr>
<td>Silvopastoral exploitation: intensive</td>
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<tr>
<td>Silvopastoral exploitation: extensive</td>
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<td>Abandoned</td>
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<tr>
<td>Rotation cultures: 9-years system</td>
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<td>Fertilizers input: 9-years ago</td>
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<td>Fertilizers input: 3-years ago</td>
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<tr>
<td>Crop production</td>
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<td>Heavy machinery last 12 months</td>
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<td>Heavy machinery 4 years ago</td>
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<tr>
<td>Heavy machinery 6 years ago</td>
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<tr>
<td>Heavy machinery 9 years ago</td>
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<tr>
<td>Shrub cutting with tractor 2 years ago</td>
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<tr>
<td>Cork oak regeneration: high</td>
<td></td>
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<tr>
<td>Cork oak regeneration: low</td>
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<tr>
<td>Cork exploitation: 9-10 years</td>
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</tr>
</tbody>
</table>
Cork quality: good

Cork quality: regular

Mushrooms sampling

| Table 3. Ectomycorrhizal fungi diversity descriptors for each stand (a and b) selected in the nine montado areas (values are means of four replicates). LT – Leitões; CÇ – Couço; FM – Freixo do Meio; SB – Sobrado; AM – Serra Mendes de Cima; ES – Espadanal do Sul; OG – Outeiro da Guarita; DA – Daroeira; VC – Vale Coentros. |
|-----------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| ECM fungi descriptors | LTa    | LTb    | CÇ     | FMa    | FMb    | SB     | AMa    | AMb    | ESa    | ESb    | OGa    | OGb    | DA     | VCa    | VCb    |
| Abundance (total tips)  | 5287   | 4955   | 7545   | 4440   | 4429   | 6155   | 8003   | 3670   | 8167   | 10603  | 4177   | 15453  | 9907   | 10755  | 11962  |
| Abundance (total ECM)   | 3834   | 3470   | 3957   | 3723   | 2609   | 3337   | 4065   | 3389   | 4965   | 5293   | 4971   | 6363   | 4688   | 4222   | 4018   |
| Abundance active ECM (%)| 32.50  | 33.00  | 43.90  | 28.40  | 26.40  | 41.60  | 47.50  | 25.40  | 40.40  | 48.00  | 18.80  | 57.30  | 54.10  | 62.30  | 72.40  |
| Number of Taxa          | 11     | 11     | 14     | 19     | 20     | 17     | 15     | 15     | 15     | 9      | 14     | 14     | 9      | 14     | 14     |
| Species diversity (Shanon) | 2.18   | 2.59   | 3.57   | 2.94   | 2.95   | 2.90   | 2.90   | 2.49   | 2.51   | 3.14   | 3.22   | 2.27   | 2.27   | 2.94   | 2.90   |
| Species Evenness (Pielou) | 0.63   | 0.75   | 0.71   | 0.84   | 0.68   | 0.72   | 0.79   | 0.74   | 0.83   | 0.79   | 0.87   | 0.72   | 0.77   | 0.76   |        |
| Log alfa index          | 1.33   | 1.34   | 1.66   | 2.55   | 2.70   | 2.13   | 1.78   | 2.00   | 0.89   | 0.97   | 1.81   | 1.52   | 0.98   | 1.61   | 1.57   |
| Jack knife index        | 10.67  | 11.00  | 14.00  | 19.00  | 21.25  | 16.67  | 15.00  | 15.00  | 15.00  | 8.00   | 9.00   | 14.00  | 9.00   | 14.00  | 14.00  |
| Species richness (Margalef) | 1.17   | 1.18   | 1.46   | 2.14   | 2.26   | 1.83   | 1.56   | 1.71   | 0.78   | 0.86   | 1.56   | 1.35   | 0.87   | 1.40   | 1.38   |
| Simpson diversity       | 3.70   | 4.88   | 4.57   | 9.44   | 4.45   | 5.91   | 6.29   | 5.55   | 4.78   | 4.23   | 6.88   | 7.39   | 3.91   | 5.57   | 5.54   |
| Beta diversity (Whittaker)| 1.00   | 1.20   | 1.55   | 0.90   | 0.74   | 1.19   | 1.50   | 1.22   | 0.39   | 0.64   | 0.75   | 1.24   | 0.71   | 0.81   | 0.75   |

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Table 4. Soil chemistry parameters and climatic conditions for each stand (a and b) selected in the nine *montado* areas. The values are presented as the average for the

LT – Leitões; CÇ – Couço; FM – Freixo do Meio; SB – Sobrado; AM – Serra Mendes de Cima; ES – Espadanl do Sul; OG – Outeiro da Guarita; DA – Daroeira;

VC – Vale Coentros.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LTa</th>
<th>LTb</th>
<th>CÇb</th>
<th>FMa</th>
<th>FMb</th>
<th>AMa</th>
<th>AMb</th>
<th>SBa</th>
<th>ESa</th>
<th>ESb</th>
<th>OGa</th>
<th>OGb</th>
<th>DAa</th>
<th>VCa</th>
<th>VCb</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (%)</td>
<td>2.23</td>
<td>1.25</td>
<td>0.99</td>
<td>1.22</td>
<td>1.51</td>
<td>2.66</td>
<td>2.62</td>
<td>2.59</td>
<td>1.22</td>
<td>1.75</td>
<td>2.81</td>
<td>2.11</td>
<td>2.11</td>
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<td>4.60</td>
<td>5.20</td>
<td>5.30</td>
<td>5.70</td>
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<td>4.50</td>
<td>5.10</td>
<td>4.90</td>
<td>5.00</td>
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</tr>
<tr>
<td>K⁺ (me/ 100g)</td>
<td>0.54</td>
<td>0.72</td>
<td>0.24</td>
<td>0.78</td>
<td>0.84</td>
<td>0.26</td>
<td>0.33</td>
<td>0.21</td>
<td>0.23</td>
<td>0.79</td>
<td>0.52</td>
<td>0.60</td>
<td>0.68</td>
<td>0.60</td>
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<tr>
<td>Na⁺ (me/ 100g)</td>
<td>0.04</td>
<td>0.10</td>
<td>0.04</td>
<td>0.11</td>
<td>0.13</td>
<td>0.09</td>
<td>0.12</td>
<td>0.05</td>
<td>0.05</td>
<td>0.17</td>
<td>0.12</td>
<td>0.11</td>
<td>0.15</td>
<td>0.11</td>
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<tr>
<td>Ca²⁺ (me/ 100g)</td>
<td>0.80</td>
<td>0.62</td>
<td>0.92</td>
<td>1.91</td>
<td>2.45</td>
<td>1.90</td>
<td>1.69</td>
<td>2.16</td>
<td>1.01</td>
<td>1.70</td>
<td>2.33</td>
<td>0.78</td>
<td>1.88</td>
<td>1.22</td>
<td>1.06</td>
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<tr>
<td>Mg²⁺ (me/ 100g)</td>
<td>0.33</td>
<td>0.80</td>
<td>0.33</td>
<td>0.60</td>
<td>0.92</td>
<td>1.24</td>
<td>0.92</td>
<td>1.08</td>
<td>0.43</td>
<td>0.52</td>
<td>1.24</td>
<td>0.60</td>
<td>1.72</td>
<td>0.84</td>
<td>0.96</td>
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<td>Average Rainfall per year (mm)</td>
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<td>18.50</td>
<td>18.50</td>
<td>54.60</td>
<td>54.60</td>
<td>54.60</td>
<td>54.60</td>
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<td>49.20</td>
<td>49.20</td>
<td>49.20</td>
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<tr>
<td>Average Rainfall Spring (mm)</td>
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<td>7.43</td>
<td>7.43</td>
<td>82.50</td>
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</tbody>
</table>
Legends

Fig. 1. Relative abundance of aECM associated to cork oak roots in the studied montado areas under different land use practices.

Fig. 2. Ectomycorrhizal fungi taxa in the nine montado areas in study (values are mean ± SD of 8 replicates, except for the montados CÇ and DA in which 4 replicates were considered), after a Newman-Keuls test (p<0.01).

Fig. 3. Total root tips (RTip) in the nine montado areas in study (values are mean ± SD of 8 replicates, except for the montados CÇ and DA in which 4 replicates were considered), after a Newman-Keuls test (p<0.01).

Fig. 4. Number of aECM associated to cork oak roots in the nine montado areas in study (values are mean ± SD of 8 replicates, except for the montados CÇ and DA in which 4 replicates were considered), after a Newman-Keuls test. (LT-montado, CÇ-montado, FM- montado, SB- montado, AM- montado, ES- montado, OG-montado, DA-montado, VC-montado).

Fig. 5. Redundancy Analysis (RDA) based on ectomycorrhizal fungi biodiversity and environmental descriptors. Significance of canonical axes: axis 1 (eigenvalue=0.343, F=4.181, p<0.01), all other axes (eigenvalue=0.8, F=5.358, p<0.01). ECM = mycorrhized tips; aECM = active ectomycorrhized tips; iECM = inactive ectomycorrhized tips; nECM = non-mycorrhized tips; %ECM = percentage of mycorrhized tips; %aECM = percentage of active ectomycorrhized tips; %iECM = percentage of inactive ectomycorrhized tips; %nECM = percentage of non-mycorrhized tips; Crop Rot_L&N = crop rotation cultures using legumes and nutrient supply; Pine = oak woodlands mixed with pine; Low_Mort = oak woodlands with low mortality of cork oak; Av_Temp = average annual temperature; Na = sodium content in soil; Mobil_0 = recent soil tillage.
Appendix

Ectomycorrhizal material. The isolated ectomycorrhizae are maintained in the Department of Botany of the University of Coimbra (COI), Portugal.

Amanita rubescens AJ889923 ( = Quercirhiza internangularis + Quercus suber; Azul et al., 2005): AAM 150/00, leg. 20.06.2000, VCb.

Boletaceae sp1 ( = Quercirhiza boletonivescens; Azul, 2002): AAM 121/00, leg. 20.07.2000, AMa.

Boletaceae sp2 ( = Quercirhiza lecciummormorpha; Azul, 2002): AAM 199/00, leg. 20.06.2000, VCa.

Cenococcum geophilum Fr. (Azul, 2002): AAM 103/00-II, leg. 20.06.2000, LTa.

Cortinariaceae sp1 ( = Quercirhiza cortinarioglutinosa; Azul, 2002): AAM 179/00, leg. 20.06.2000, OGb.

Cortinariaceae sp2 ( = Quercirhiza cortinariolanata; Azul, 2002): AAM 195/00, leg. 20.06.2000, ESA.

Cortinariaceae sp3 ( = Quercirhiza argenteoalba; Azul, 2002): AAM176/00-I, leg. 20.07.2000, FMa.

Cortinariaceae sp4 ( = Quercirhiza argenteoinflata; Azul, 2002): AAM 140/00-I, leg. 20.06.2000, ESA.

Cortinariaceae sp5 ( = Quercirhiza argenteozonata; Azul, 2002): AAM 102/00-II, leg. 20.06.2000, OGa.

Genea sp1 ( = Quercirhiza geneanitida; Azul, 2002): AAM 133/00-II, leg. 20.06.2000, ESA.

Lactarius chrysorrheus (Fr.) Fr. AF09698342 (Azul, 2002): AAM 117/00-II, leg. 20.06.2000, LTa.

Lactarius sp1 ( = Quercirhiza lactofragilis; Azul, 2002): AAM 118/00-II, leg. 20.06.2000, CCb.

Lactarius sp2 ( = Quercirhiza lactoflavescens; Azul, 2002): AAM 186/00, leg. 20.06.2000, OGa.

Lactarius sp3 ( = Quercirhiza lactonitida; Azul, 2002): AAM 118/00-II, leg. 20.06.2000, CCb.


Lactarius sp5 ( = Quercirhiza russulodecantata; Azul, 2002): AAM 141/00-II, leg. 20.06.2000, LTa.

Lactarius sp6 ( = Quercirhiza russuloepidermata; Azul, 2002): AAM 143/00, leg. 20.06.2000, OGa.

Lactarius sp7 ( = Quercirhiza russuloespamerata; Azul, 2002): AAM 225/00, leg. 20.06.2000, LTb.

Lactarius sp8 AF230897 ( = Quercirhiza russulopallida; Azul, 2002): AAM 190/00, leg. 20.06.2000, OGa.

Lactarius sp9 ( = Quercirhiza russulospinosa; Azul, 2002): AAM 196/00, leg. 20.06.2000, ESA.

Lactarius sp10 ( = Quercirhiza russulosplendida; Azul, 2002): AAM 149/00, leg. 20.07.2000, AMa.

Lactarius sp11 AF418615 ( = Quercirhiza russulovillosa; Azul, 2002):


Figure 1
Figure 2
Figure 3
Figure 4
Figure 5