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Land use practices and ectomycorrhizal fungal communities from oak woodlands dominated by *Quercus suber* L. considering drought scenarios

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Abstract Oak woodlands in the Mediterranean basin have been traditionally converted into agro-silvo-pastoral systems and exemplified sustainable land use in Europe. In Portugal, in line with the trend of other European countries, profound changes in management options during the twentieth century have led to landscape simplification. Landscapes are dynamic and the knowledge of future management planning combining biological conservation and soil productivity is needed, especially under the actual scenarios of drought and increasing evidence of heavy oak mortality. We examined the ectomycorrhizal (ECM) fungal community associated with cork oak in managed oak woodlands (called *montado*) under different land use practices, during summer. ECM fungal richness and abundance were assessed in 15 stands established in nine *montados* located in the Alentejo region (southern Portugal), using morphotyping and ITS rDNA analysis. Parameters related to the *montados* landscape characteristics, land use history over the last 25 years, climatic and edaphic conditions were taken into account. Fifty-five ECM fungal taxa corresponding to the most abundant fungal symbionts were distinguished on cork oak roots. *Cenococcum geophilum* and the families *Russulaceae* and *Thelephoraceae* explained 56% of the whole ECM fungal community; other groups were represented among the community: *Cortinariaceae*, *Boletaceae*, *Amanita*, *Genea*, *Pisolithus*, *Scleroderma*, and *Tuber*. There were pronounced differences in ECM fungal community structure among the 15 *montados* stands: *C. geophilum* was the only species common to all stands, tomentelloid and russuloid species were detected in 87–93% of the stands, *Cortinariaceae* was detected in 60% of the stands, and the other groups were more unequally distributed. Ordination analysis revealed that ECM fungal richness was positively correlated with the silvo-pastoral exploitation regime and low mortality of cork oak, while ECM fungal abundance was positively correlated with extensive agro-silvo-pastoral exploitation under a traditional 9-year rotation cultivation system and recent soil tillage. The effects of land use on the ECM fungal community and its implications in different scenarios of landscape management options, oak mortality, and global warming are discussed.

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Introduction

Management planning 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). Large areas of southern Iberian Peninsula (ca. 6.5 million ha; DGF 2003) correspond to an open savannah-like landscape, called *montado* in Portugal, which results from an extensive agro-silvo-pastoral exploitation. *Montados* dominate the landscape of the Alentejo region (southern Portugal). These ecosystems consist of open oak formations (usually *Quercus suber* L., *Quercus rotundifolia* L., and *Quercus ilex* L.) with scattered tree cover (60–100 trees per ha) and one or two vegetation strata at the ground level, composed of shrubs and/or herbaceous plants (natural pastures and crops), in a 9–year rotation cultivation system (Pinto-Correia 1993b). Animals (sheep, iberian pig, black goat, and/or cattle) are kept in the *montado* all year round to preserve natural renovation. Every 6–8 years, depending on the soil quality, livestock is moved to other pastures, and the soil is cultivated for 1 or 2 years (commonly the first year with wheat, the second with oat, rye, or barley). Crop cultivations are the less important part of the exploitation, but an efficient measure against the development of shrubs and an important complement in the livestock nourishment (Pinto-Correia and Mascarenhas 1999). Traditionally, those 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 and represent a classic example of sustainable land use in Europe by combining biological conservation and socio-economical value (Joffre et al. 1999; Pinto-Correia 1993b; Pulido et al. 2001; Pinto-Correia and Vos 2004). However, successive disturbances over the second half of twentieth century, through both intensification of land use practices and extensification, have dramatically changed the *montado* landscape (Pinto-Correia and Mascarenhas 1999). Large areas of oak were abandoned and underwent the natural process of ecological succession (Pinto-Correia 1993b; Debussche et al. 1999; Pinto-Correia and Vos 2004). Through this process, managed areas were progressively re-colonized by autochthonous vegetation, changing from herbaceous dominancy or silvo-pastoral systems with shrubs artificially maintained in low densities to shrub-dominated areas. Such reestablishment of shrublands increases the risk of large fires (Nunes et al. 2005), plant diseases (Brasier 1996), and over-exploitation of soil resources (Pinto-Correia and Mascarenhas 1999), which negatively affect plant community resilience (Díaz-Delgado et al. 2002). In the Alentejo region, 65% of soil is classified as having low capacity for agriculture or not suitable for any economic use, with high erosion risk (Pinto-Correia 1993a); the decline of *montado* in this region has further led to the loss of microbial, plant and animal diversity, and productivity (Hector et al. 1999; Da Silva et al. 2008). Changes in management planning and landscape simplification are largely extended to other European countries (Díaz-Delgado et al. 2002; Mouillot et al. 2005; Rios-Díaz et al. 2006). In Mediterranean basin, the situation is even more critical due to the actual scenarios of climate warming (European Environment 2004) and increasing evidence of heavy mortality of evergreen oaks: *Q. suber* and *Q. rotundifolia* (Brasier 1996; Brasier and Scott 2008).

Ectomycorrhizal (ECM) fungal species and fungal networks are widely recognized as mediators between soil processes and plant community, by enhancing nutrient acquisition, drought tolerance, and pathogen resistance of their hosts (Perez-Moreno and Read 2000; Högberg and Högberg 2002; Read and Perez-Moreno 2003; Leake et al. 2004; Selosse et al. 2006; Smith and Read 2008), thus influencing seedling establishment, plant diversity, and vegetation community dynamics. Previous studies revealed that the ECM fungal community in Portuguese *montado* seem to be quite diverse in composition and structure (Azul et al. 1999; Azul et al. 2001a, b, c, d, e; Azul 2002; Azul et al. 2006a, b, c, d, e, f, g, h; Azul et al. 2008a, b, c, d), but it remains unclear which factors influence the establishment of ECM fungal species and implications for the soil processes, particularly during drought conditions.

Different types of management affect soil productivity and conservation and overall ecosystem health. Recent studies have shown that traditional management in Mediterranean basin create low levels of disturbance and enhance the diversity of flora and fauna; agro-silvo-pastoral landscapes can support a greater overall biodiversity than climax European woodlands from which they first have derived (Decocq et al. 2004; Pinto-Correia and Vos 2004; Rios-Díaz et al. 2006). However, little is known about the influence of land use on soil micobiota in managed oak woodlands (Azul 2002; Azul et al. unpublished data).

The objectives of the present study were to examine the ECM fungal community in Portuguese *montados* during summer - a critical period under Mediterranean climate influence -, to explore the relationships between the ECM fungal community structure and multiple explanatory data, and to look into its possible implications in management planning options. We have compiled information related to the *montado* landscape characteristics, land use history over the past 25 years, climatic and edaphic conditions, and we combined these qualitative data with ECM fungal diversity descriptors.

Materials and methods

Study site and experimental design

The study was conducted in nine *montados* located in the leading cork producing area of the country: Alentejo, southern Portugal (Fig. 1). All *montados* correspond to a savannah-like landscape consisting of open oak formations under different land use practices. The *montados* areas were chosen in close collaboration with farmers, land users, and/or stakeholders and are representative of the main landscape management practices in the region (Table 1).

Two stands (a and b) were selected per *montado*, except for DA-*montado*, SB-*montado*, and CÇ-*montado*. Fifteen stands (50 m × 100 m) were established. The two stands from the same *montado* were selected 1 km apart from each other in order to be independent. Information related to the *montado* landscape characteristics and land use history over the last 25 years was obtained by interviewing farmers, land users, and/or stakeholders. The questionnaire used in the interviews is presented in the Appendix (A1) and the results are summarized in Table 2. Vegetation surveys in *montado* stands measured the dominant species at the peak of vegetation cover, from May to July 2000 (Table 1). Soil sampling was performed during summer, between June and August 2000. Soil K, Na, Ca, and Mg were determined after an extraction with ammonium acetate at pH7 (Chapman 1979). Soil organic matter was determined by the Tinsley method (LQARS 1977); pH was measured on 2.5:1 distilled water/soil suspension (LQARS 1977).

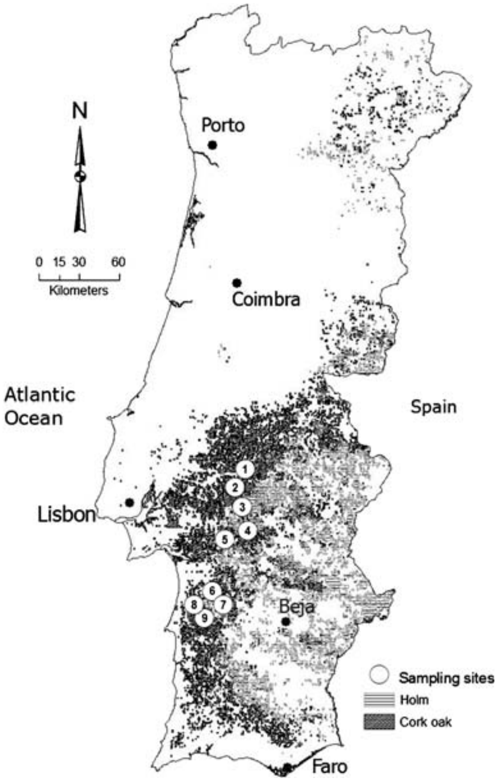


Fig. 1 Location of the nine *montados* in the Alentejo region (southern Portugal). 1 Ponte Sôr (Leitões, LT-*montado*), 2 Coruche (Couço, CÇ-*montado*), 3 Montemor-o-Novo (Freixo do Meio, FM-*montado*), 4 Alcácer do Sal (Sobrado, SB-*montado*), 5 Alcácer do Sal (Serra Mendes de Cima, AM-*montado*), 6 Grândola (Espadanal do Sul, ES-*montado*), 7 Santiago do Cacém (Outeiro da Guarita, OG-*montado*), 8 Santiago do Cacém (Daroeira, DA-*montado*), 9 Grândola (Vale Coentros. VC-*montado*)

Sampling and assessment of ECM

Four cork oak trees were selected at 25-m intervals in each *montado* stand. For each tree, four 10 × 10 × 15 cm monoliths of soil were collected, one along each cardinal directions (N, E, S, W). Rhizospheric soil was taken at 60 cm from the trunk to ensure that roots belonged to the selected tree. The four monoliths per tree were wrapped separately in wet newspaper and sealed in plastic bags. In the laboratory, the four monoliths per tree were pooled, and one 1,500-cm^3 sample of crumbled soil with roots was considered for the ECM assessment. The selected material was stored at 4°C for up to 2 weeks until being processed. Soil particles adhering to roots were removed in water with pipettes, fine forceps, and preparation needles, under a Wild Heerbrugg stereomicroscope. A total of 260,545 cork oak root tips (RTip) were analyzed and sorted into mycorrhizal (ECM) and non-mycorrhizal tips (nECM) by the presence of mantle. Mycorrhizal tips were then separated into active and inactive tips (aECM and iECM, respectively); ECM tips with a wrinkled mantle were considered as inactive tips. The living ECM roots were categorized into morphotypes according to mantle characteristics and 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; Suz et al. 2008). The morphotypes were previously described in detail (Azul 2002), and some of the new ECM species have been published (Azul et al. 2001a, b, c, d, e; Azul et al. 2006a, b, c, d, e, f, g, h; Azul et al. 2008a, b, c, d). Further identification of the ECM fungal species was done through ITS rDNA analysis. Detailed descriptions of all aECM, photos, drawings, and slides are available from the corresponding author. A subsample of each ECM morphotype was preserved in alcohol 50% and in 2% CTAB as a voucher and kept in the Department of Botany of the University of Coimbra (comp. Appendix, Table 3).

DNA analysis

The identification of some ECM material was possible using the following protocols: fungal DNA MiniPrep Kit (Omega, Biotech, Doraville, USA) was applied and ITS nrDNA was amplified by nested PCR, using the primer pair ITS1F/ITS4B (Gardes and Bruns 1983) in the first amplification and ITS1/ITS4 (White et al. 1990) in the

Table 1 Location and characterization of vegetation cover in the 15 *montados* stands

Study site/*montado* area	Location	Vegetation cover
Ponte Sôr–Leitões (LT)	39°08′ 02″ N, 8°12′ 36″ W	a, b-*Quercus suber* L. (canopy 30%) + *Pinus pinaster* Aiton (canopy 20%); shrubs 25% density, mainly *Cistus salvifolius* L.
Coruche–Couço (CÇ)	38°56′ 25″ N, 8°18′ 11″ W	*Q. suber* (canopy 60–80%), shrubs 50% density (*Cistus salvifolius*, *Cistus crispus* L., and *Lavandula* sp.)
Montemor-o-Novo–Freixo do Meio (FM)	38°41′ 10″ N, 8°20′ 23″ W	a, b-*Q. suber* (canopy 50%); shrubs 25–35% density (*C. ladanifer* L., *C. salvifolius*, *C. crispus*, and *Genista triacanthus* Brot.)
Alcácer do Sal–Serra Mendes de Cima (AM)	38°32′ 02″ N, 8°23′ 17″ W	a-*Q. suber* (canopy 70%), shrubs 5–10% canopy, mainly *C. salvifolius.* b-*Q. suber* (canopy 80%); shrubs 90% density (*Calluna vulgaris* (L.) Hull, *Cistus ladanifer*, *G. triacanthus*, *Lavandula* sp., and *Ulex* sp.)
Alcácer do Sal–Sobrado (SB)	38°32′ 02″ N, 8°23′ 17″ W	*Q. suber* (canopy 50%), *Q. rotundifolia* L. (canopy 10%); shrubs 5–10% density (*Cistus* sp., *G. triacanthus* and *Lavandula* sp.)
Grândola–Espadanal do Sul (ES)	38°11′ 45″ N, 8°39′ 13″ W	a, b-*Q. suber* (canopy 50%) + *Pinus pinea* L. (vestigial); shrubs 5% density (*Cistus salvifolius*, *Halimium* sp., *G. triacanthus*, *Chamaeaspartum tridentatum* L., and *Ulex* sp.)
Santiago do Cacém–Outeiro da Guarita (OG)	38°13′ 24″ N, 8°28′ 19″ W	a-*Q. suber* (canopy 20%) and *Pyrus bourgaeana* Decen (vestigial); shrubs 25–35% density (*Arbutus unedo* L., *Cistus populifolius* L., *C. ladanifer*, *C. salvifolius*, and *G. triacanthus*). b-*Q. suber* (canopy 80%), shrubs 60–70% vegetation cover (*C. crispus*, *C. ladanifer*, *C. salvifolius*, *Lavandula pedunculata* Miller., *Lavandula* sp., *G. triacanthus*, and *Ulex* sp.)
Santiago do Cacém–Daroeira (DA)	38°13′ 32″ N, 8°29′ 03″ W	*Q. suber* (canopy 50%), shrubs 95% density, mainly *C. ladanifer*
Grândola–Vale Coentros (VC)	38°03′ 37″ N, 8°33′ 41″ W	a, b-*Q. suber* (canopy 20%), shrubstrata absent due to cutting practices followed soil tillage

second amplification (nested PCR). First PCR reactions were done by 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/l. Ten to fifteen ECM tips per morphotype were used. In the nested PCR, 1 µl of the first amplification was used as DNA template. The PCR product was purified using QIAquick Gel PCR purification kit (QIAGEN, 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). Sequencher (Gene Codes, 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 (Table 3 in the Appendix). Nucleotide BLASTN searches (Altschul et al. 1997) with option Standard nucleotide BLAST of BLASTN 2,6 were used to compare the sequence obtained against the sequences in the National Center of Biotechnology Information nucleotide databases.

Data analysis

ECM fungal community diversity was described by using the parameters RTip (root tips), ECM (mycorrhizal tips), nECM, aECM, and iECM. The RTip was assessed by the cumulative number of the total root tips, i.e., RTip=aECM+iECM+nECM (Azul 2002). Within the aECM, the relative abundance of a given morphotype X(X-ECM) was defined as the cumulative number of active ectomycorrhizae of type X, divided by total aECM on sample (Azul 2002).

A nested ANOVA (Zar 1996) was used to compare all above parameters between the 15 stands and between the nine *montados*. Normality and homogeneity of variance 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 (1996) recommendations. ECM diversity at a given study stand was estimated by the following descriptors: (a) species richness, i.e., number of ECM species found per stand (*S*); (b)

Table 2 ECM fungal descriptors, soil characterization (values are means of four replicates), *montado* landscape characteristics, and land use history over the last 25 years for each *montado* stand

	LTa	LTb	CÇb	FMa	FMb	AMa	AMb	SBa	ESa	ESb	OGa	OGb	DAa	VCa	VCb
ECM fungal descriptors															
Abundance aECM	5,287	4,955	7,545	4,440	4,429	6,155	8,003	3,670	8,167	10,603	4,177	15,453	9,907	10,755	11,962
Number of taxa	11	11	14	19	20	17	15	15	8	9	14	14	9	14	14
Species diversity (Shannon)	2.18	2.59	2.71	3.57	2.94	2.95	3.10	2.90	2.49	2.51	3.14	3.22	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	0.82	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
Soil composition															
C (%)	2.23	1.25	0.99	1.22	1.51	2.66	2.62	2.59	1.22	1.75	2.81	2.11	2.11	2.11	2.19
pH	4.30	4.60	5.20	5.30	5.70	5.10	5.20	5.30	5.10	5.00	5.30	4.50	5.10	4.90	5.00
K^+ (me/100 g)	0.54	0.72	0.24	0.78	0.62	0.84	0.26	0.33	0.21	0.23	0.79	0.52	0.60	0.68	0.60
Na^+ (me/100 g)	0.04	0.10	0.04	0.11	0.07	0.13	0.09	0.12	0.05	0.05	0.17	0.12	0.11	0.15	0.11
Ca^{2+} (me/100 g)	0.80	0.62	0.92	1.91	2.45	1.90	1.69	2.16	1.01	1.70	2.33	0.78	1.88	1.22	1.06
Mg^{2+} (me/100 g)	0.33	0.80	0.33	0.60	0.92	1.24	0.92	1.08	0.43	0.52	1.24	0.60	1.72	0.84	0.96
Average rainfall per year (mm)	18.50	18.50	18.50	54.60	54.60	54.60	54.60	54.60	49.20	49.20	49.20	49.20	49.20	49.20	49.20
Average rainfall spring (mm)	7.43	7.43	7.43	82.50	82.50	82.50	82.50	82.50	64.50	64.50	64.50	64.50	64.50	64.50	64.50
Average temperature per year (°C)	16.00	16.00	16.00	16.40	16.40	16.40	16.40	16.40	16.20	16.20	16.20	16.20	16.20	16.20	16.20
Average temperature spring (°C)	16.80	16.80	16.80	16.40	16.40	16.40	16.40	16.40	16.40	16.40	16.40	16.40	16.40	16.40	16.40
Montado landscape characteristics/Land use history															
Montado landscape/ha	741.2 Oak-pine		227 Oak	1,100 Oak		111 Oak		15 Oak	45 Oak		36 Oak		37 Oak	23 Oak	
Land exploitation	C		C	C		F		F	F		F		F	F	
Exploitation regime	$E_{silvpast}$	$E_{silvpast}$	$E_{silvpast}$	$E_{silvpast}$	$E_{silvpast}$	$E_{silvpast}$	None	$I_{silvpast}$	$E_{agsilvpast}$	$E_{agsilvpast}$	$E_{silvpast}$	$E_{silvpast}$	None	$I_{silvpast}$	$I_{silvpast}$
Cork oak canopy: trees/ha	45	10	40	53	53	30	60	38	30	15	60	60	70	60	60
Cork oak mortality: trees/ha	1.3	2	1.2	0.3	0.3	0	0	0.2	2.5	2.5	0.2	0	1.5	0.5	0.5
Shrubs density cover (%)	≤35	≤35	±50	≤35	≤35	≤35	≥90	≤35	±50	±50	±50	±50	≥90	≤35	≤35
Shrub density control	Cut	Cut	Cut	Cut	Cut	Cattle	0	Cattle	Cattle	Cattle	Cut	$Soil_{tillage}$	0	$Soil_{tillage}$	$Soil_{tillage}$
Last shrub control/years	6y	6y	4y	6y	6y	9y	>9y	9y	9y	9y	4y	4y	>15y	<1y	<1y

Table 2 (continued)

	LTa	LTb	CÇb	FMa	FMb	AMa	AMb	SBa	ESa	ESb	OGa	OGb	DAa	VCa	VCb
Montado landscape characteristics/Land use history															
Last soil tillage/years	9y	9y	9y	9y	9y	9y	>25y	6y	4y	4y	6y	4y	>25y	<1y	<1y
Last fertilization/years (F)	F_{9y}	$F_{>9y}$	F_{9y}	F_{9y}	F_{9y}	F_{9y}	$F_{>9y}$	F_{9y}	F_{3y}	F_{3y}	F_{9y}	F_{9y}	$F_{>9y}$	F_{9y}	F_{9y}
Cork oak regeneration (R)	R_{low}	R_{low}	R_{low}	R_{low}	R_{high}	R_{high}	R_{high}	R_{low}	R_{low}	R_{low}	R_{high}	R_{low}	R_{low}	R_{low}	R_{low}
Cork harvesting (H)	H_{9y}	H_{9y}	H_{9y}	H_{9y}	H_{9y}	H_{9y}	H_{9y}	H_{9y}	H_{9y}	H_{9y}	H_{9y}	H_{9y}	H_{9y}	H_{9y}	H_{9y}
Cork quality (CQ)	CQ_{good}	CQ_{good}	CQ_{good}	CQ_{good}	CQ_{reg}	CQ_{good}	CQ_{good}	CQ_{reg}	CQ_{reg}	CQ_{reg}	CQ_{good}	CQ_{good}	CQ_{reg}	CQ_{good}	CQ_{good}
Mushrooms sampling (Mush)	$Mush_y$	$Mush_y$	$Mush_y$	$Mush_{no}$	$Mush_{no}$	$Mush_{no}$	$Mush_{no}$	$Mush_y$	$Mush_y$	$Mush_y$	$Mush_y$	$Mush_y$	$Mush_{no}$	$Mush_y$	$Mush_y$

LT, CÇ, FM, SB, AM, ES, OG, DA, and VC *montados* identification (abbreviations as in Fig. 1). Abundance aECM = cumulative number of aECM associated to cork oak roots, present over 5% of total aECM; Number of Taxa = cumulative number of ectomycorrhizal fungal taxa. Oak = cork oak woodlands; Oak_{pine} = cork oak woodlands mixed with pine; $E_{silvpast}$ = extensive silvopastoral exploitation; $I_{silvpast}$ = intensive silvopastoral exploitation; $E_{agsilvpast}$ = extensive agro-silvo-pastoral exploitation; = Cu shrub density controlled by using cutting practices with no soil tillage; Ca = shrub density controlled by permanent grazing of sheep; $Soil_{tillage}$ = shrub density controlled by using cutting practices followed soil tillage; F_{xy} = last use of fertilizers (*x* means the number of years); R_{low} = low cork regeneration; R_{high} = high cork regeneration; H_{xy} = last cork harvesting (*x* means the number of years); CQ_{good} = cork quality good; Q_{reg} = cork quality regular; $Mush_y$ = mushroom collection; $Mush_{no}$ = no mushroom collection

Shannon–Wiener (*H*) and Simpson (λ) diversity indexes; (c) Pielou evenness (*H′*); (d) *Margalef* (D), Log α (*S*) and Jack Knife richness indexes; and (e) Whittaker β-diversity index (Magurran 1988). The relationships between ECM diversity descriptors and environmental variables (coded from Table 2) were done using a redundancy analysis (RDA). A forward selection procedure was performed to determine those explanatory environmental 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 analysis was performed using STATISTICA 6.0 software package (StatSoft 2001), and multivariate analysis was done using the CANOCO 4.5 software (Ter Braak and Smilauer 2002). Data analysis was applied to the most abundant aECM taxa, present over 5% of total aECM per 1,500 cm³ sample.

Results

ECM fungal community structure

Fifty-five ECM fungal taxa were distinguished according to ectomycorrhizae morphology/anatomy and supplemented with ITS rDNA analysis (accession numbers in the Appendix, Table 3). Particular attention was given to the most abundant aECM taxa (present over 5% of total aECM per sample) and to its structural features (ECM/reference in Appendix, Table 3), but many rare species were observed.

When considering the ECM fungal community composition as a whole, the species *Cenococcum geophilum* and the families *Russulaceae* and *Thelephoraceae* represented altogether 56% of the mycorrhizas examined. The genera *Russula* and *Tomentella* were the richest in species, with 13 and 15 taxa, respectively. Other ECM fungal groups were represented as follows: *Cortinariaceae*, five taxa; *Boletaceae*, two taxa; *Lactarius*, four taxa; *Amanita*, two taxa; *Genea*, one taxon; *Pisolithus*, one taxon; *Scleroderma*, one taxon; and *Tuber*, one taxon (see in Appendix, Table 3).

The aECM taxa exhibited high variation among the 15 stands. Figure 2 shows the abundance of aECM genera and families regarding the most abundant aECM taxa. The genus *Russula* was highly represented within ECM fungal community being present in all stands, except for ESb-*montado*; the second genus most represented, *Tomentella*, was absent in OGb-*montado* and DA-*montado*; the family *Cortinariaceae* was recorded in nine stands (LTb-*montado*, FMa,b-*montado*, SB-*montado*, AMa-*montado*, ESa,b-*montado*, and OGa,b-*montado*); the genus *Amanita* was detected in 11 stands (LTb-*montado*, CÇ-*montado*, FMa,b-*montado*, SB-*montado*, AMa,b-*montado*, ESb-*montado*, OGa-*montado*, VCa,b-*montado*) but less abundant on root tips; the family *Boletaceae* and the genus *Genea*, *Lactarius*, *Pisolithus*, *Scleroderma*, and *Tuber* were more irregularly represented and distributed (Fig. 2). In general, the same aECM taxa were rarely encountered in many stands. *C. geophilum* was the only ECM fungal species common to all stands.

ECM fungal richness and abundance

A maximum of 19–20 aECM taxa were observed in the FMa,b-*montado* under extensive silvo-pastoral exploitation, with shrubs (*Cistus salvifolius*, *Halimium* sp., *Genista triacanthus*, *Chamespartum tridentatum*, and *Lavandula* sp.) artificially maintained at 25–35% of density cover by using machinery with no soil tillage (Table 2). Higher values were also observed in SB-*montado* (15 aECM taxa) and in AMa,b-*montado*, with 17 and 15 aECM taxa, all three under silvo-pastoral exploitation with intensive and extensive regimes, respectively. The lowest values, eight and nine aECM taxa, were observed in ESa,b-*montado*, under extensive agro-silvo-pastoral exploitation ($E_{agsilvpast}$), and in DA-*montado*, that stopped being managed 15 years ago (Table 2). The variation pattern observed in the cumulative number of aECM taxa between the *montados* stands is reflected in the species richness (Margalef) values, with a clear decrease in both stands of ES-*montado*, in DA-*montado*, and in both stands of LT-*montado*, with cork oak mixed with pine (pine; *Pinus pinaster*) under extensive silvo-pastoral exploitation.

Significant changes in aECM abundance were detected amongst the 15 stands ($F_{0.05(1)8,6} = 5.45$; $p<0.05$; Fig. 3). The highest values of total aECM abundance were observed in OGb-*montado* (under extensive silvo-pastoral exploitation with last soil intervention to control shrub density applied 4 years ago), in VCa,b-*montado* (under intensive silvo-pastoral exploitation with recent shrub management followed by soil tillage), and in ESb-*montado* (under extensive agro-silvo-pastoral exploitation with the last soil intervention 4 years ago; Fig. 3). The aECM abundance was more variable than aECM richness, and no correlation was obtained between aECM abundance and aECM richness ($r=-0.33$; $p>0.05$).

Relationships between ECM fungal diversity descriptors and environmental variables

The results from the RDA showed that ECM fungal diversity descriptors were significantly related to some explanatory data mentioned in Table 2 regarding *montado* landscape characteristics, land use history over the last 25 years, and climatic and edaphic conditions. After the forward selection procedure, six variables were selected (all with $p<0.05$ after the Monte-Carlo test): agro-silvo-pastoral exploitation regime ($E_{agsilvpast}$), shrub management with recent soil tillage ($Soil_{tillage<1y}$), *montado* landscape of cork oak mixed with pine (Oak-pine), low cork oak mortality (Low_Mort), sodium content in soil (Na), and average annual temperature (Av_temperature; Fig. 4). These six variables explained 80% of the variation in ECM fungal 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 the six environmental variables and the ECM fungal diversity descriptors ($F=4.181$, $p<0.01$ for the first axis; $F=5.348$, $p<0.01$ considering all axes together).

A comparison of soil chemistry among the 15 stands was done in the forward selection procedure of the RDA. Sodium represented the only significant variable (Fig. 4), despite the differences observed (Table 2): pH values were lower in LTa,b-*montado*; *K* values were lower in AMb-*montado*, in CÇ-*montado*, and in ESa,b-montado; Ca and Mg exhibited higher variation among all stands.

The ordination diagram derived from RDA (Fig. 4) demonstrated that low cork oak mortality (Low_Mort) is a key feature affecting ECM fungal richness below-ground (Shannon index). *Montados* lacking cork oak mortality (FM, AM), or presenting low mortality rates (SB, OG, VC), exhibited higher ECM fungal richness and diversity (Table 2). ECM fungal richness and diversity were negatively correlated with management planning involving extensive agro-silvo-pastoral exploitation ($E_{agsilvpast}$; ES-*montado*). For those *montados* under silvo-pastoral exploitation regime, ECM fungal richness and diversity was negatively correlated with recent soil tillage applied in shrub management ($Soil_{tillage<1y}$; VC-*montado*) and *montado* landscape of cork oak mixed with pine (Oak-pine; LT-*montado*). The climatic conditions, here represented by the average annual temperature (Av_Temp), exhibited a positive correlation with ECM fungal community richness (Fig. 4). Concerning to the ECM fungal abundance, positive correlations were observed with extensive agro-silvo-pastoral exploitation regime ($E_{agsilvpast}$; ES-*montado*) and with silvo-pastoral exploitation regime with recent soil tillage applied in shrub management ($Soil_{tillage<1y}$; VC-*montado*; Fig. 4).

Discussion

In the present study, we determined the ECM fungal community in 15 stands of *montado* under different land use

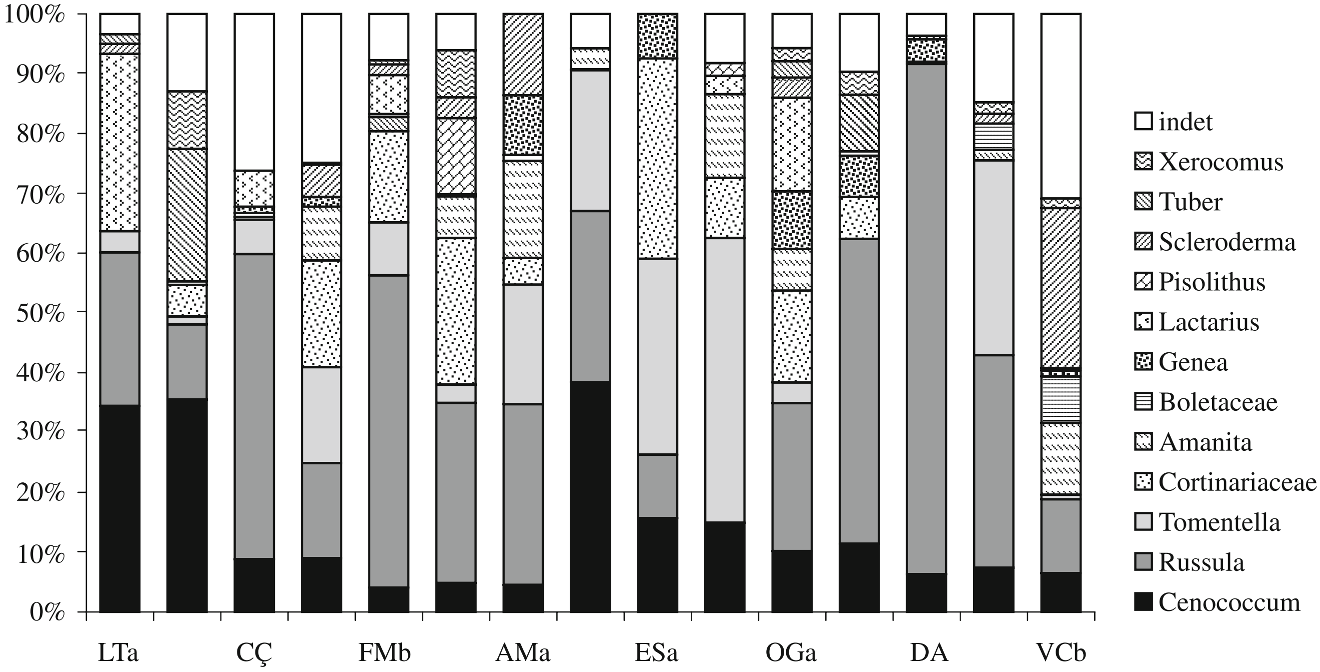
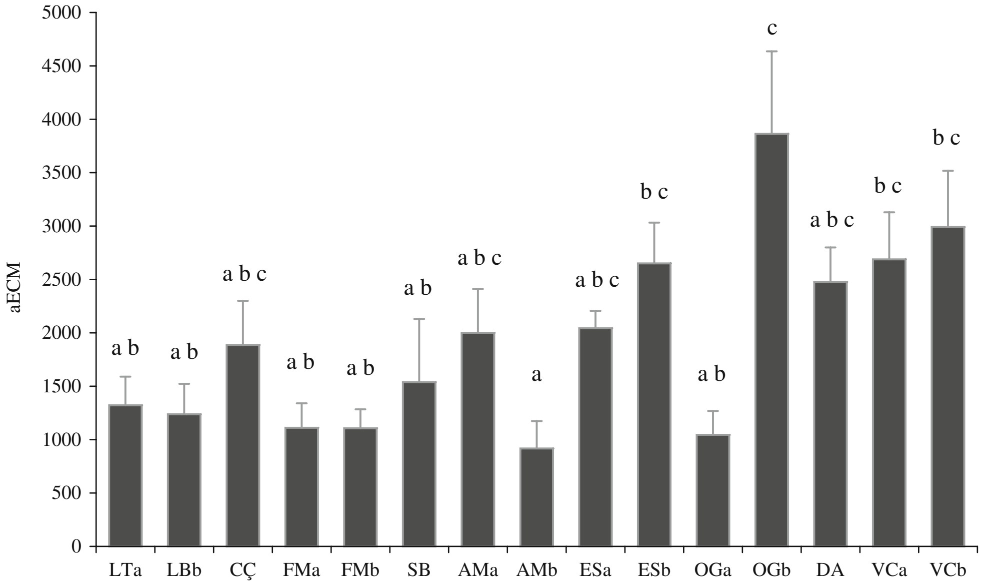


Fig. 2 Relative abundance of the most abundant ECM fungal species, genera, and families in the 15 *montados* stands under different land use practices (values are mean±SD of four replicates), after a Newman–Keuls test ($p<0.01$). The family *Boletaceae* does not include the genus *Xerocomus*. Abbreviations as in Fig. 1

practices in the Alentejo region (southern Portugal; Fig. 1). Fifty-five ECM fungal taxa were distinguished, representing the most abundant fungal symbionts on cork oak roots during the summer period. The ECM community was dominated by *C. geophilum*, *Russulaceae*, and *Thelephoraceae*. Similar results were observed in an old-growth *Q. ilex* forest in Corsica (Richard et al. 2005), in coniferous forests from the northern hemisphere (Gardes and Bruns 1996; Horton and Bruns 1998; Tedersoo et al. 2003; Lilleskov et al. 2004; Baier et al. 2006), and in a temperate oak forest in northeastern France (Courty et al. 2008). *C. geophilum* and thelephoroid ECM were among the most frequent taxa observed over a 3-year period conducted in a managed *Q. ilex* forest from the northern Iberian Peninsula (De Román and De Miguel 2005).

C. geophilum was the only species detected in all *montados* studied (Fig. 2). *Thelephoraceae* (15 taxa) and *Russula* (13 taxa), the two groups richest in species, were detected in 13 and 14 stands, respectively. *Cortinariaceae* (five taxa) and *Amanita* (two taxa) were less abundant and

Fig. 3 Abundance of aECM associated to cork oak roots in the 15 *montados* stands (values are mean±SD of four replicates), after a Newman–Keuls test. Abbreviations as in Fig. 1



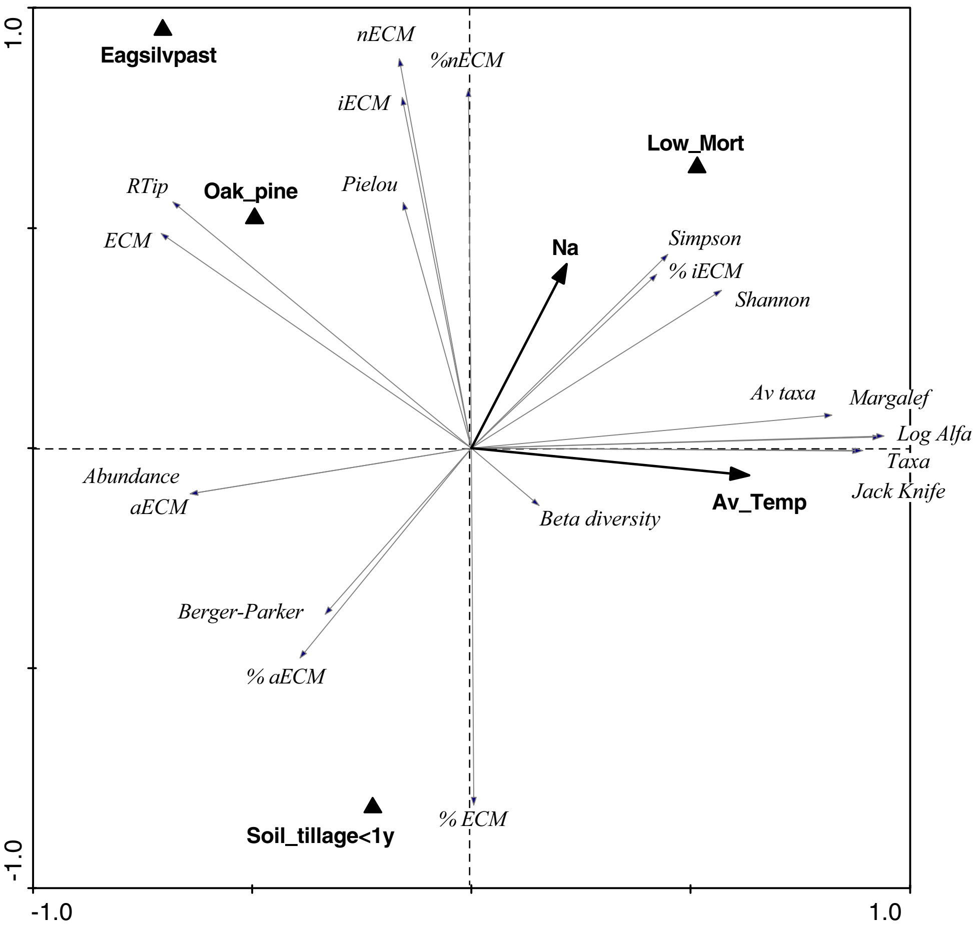


Fig. 4 Redundancy analysis based on ectomycorrhizal fungal diversity and *montado* environmental descriptors. Significance of canonical axes: axis 1 (eigenvalue=0.343, *F*=4.181, *p*<0.01), all other axes (eigenvalue=0.8, *F*=5.358, *p*<0.01). *ECM* mycorrhizal tips, *aECM* active ectomycorrhizal tips, *iECM* inactive ectomycorrhizal tips, *nECM* non-mycorrhizal tips, *%ECM* percentage of mycorrhizal tips, *%aECM* percentage of active ectomycorrhizal tips, *%iECM* percentage of inactive ectomycorrhizal tips, *%nECM* percentage of non-mycorrhizal tips, *Berger-Parker* Berger–Parker index, *Jack Knife* Jack Knife index, *Margalef* Margalef index, *Pilou* Pilou index, *Shannon* Shannon index, *Simpson* Simpson index, *Av taxa* average taxa, *Taxa* taxa number, $\text{E}_{agsilvpast}$ extensive agro-silvo-pastoral exploitation, Oak-pine *montado* of cork oak mixed with pine, Low_Mort *montados* with low mortality of cork oak, Av_Temp average annual temperature, Na sodium content in soil, Soil_tillage<1y recent soil tillage

more unequally dispersed. ECM species of *Boletaceae*, *Lactarius*, *Genea*, *Pisolithus*, *Scleroderma*, and *Tuber* were disproportionate in number of species, abundance, and distribution (Fig. 2). Earlier studies conducted in Portuguese *montados* (Azul et al. 1999; Azul et al. 2001a, b, c, d, e; Azul 2002; Azul et al. 2006a, b, c, d, e, f, g, h; Azul et al. 2008a, b, c, d) showed that the ECM fungal community is quite diverse in species and structure, but it is clearly influenced by the sampling season. Azul (2002) reported an apparent turnover in the ECM fungal community composition particularly from spring to summer; the lowest values of ECM fungal richness and abundance were observed during autumn. Seasonal fluctuations were also reported in a temperate oak forest (Courty et al. 2008) and in a managed *Q. ilex* forest, with a significant decrease in ECM fungal richness during summer (De Román and De Miguel 2005). Temporal changes of the ECM species may be explained by several factors, such as root longevity, competition for resources, and resistance to environmental variation (Courty et al. 2008), in which mycorrhizas structure, i.e., mantle organization, rhizomorphs, and cystidia, may be decisive for ecophysiological performance (Agerer 2001).

In our study, we observed that current land use practices that maintain shrubs at lower densities in *montados* (permanent grazing, cutting practices with no/followed by soil tillage) affect the composition of the ECM fungal community. ECM fungal diversity was highest in stands with extensive silvo-pastoral exploitation, with shrub controlled by cutting practices with no soil tillage and/or permanent grazing (see Table 2). This trend is important because sustainability is strongly dependent on both abiotic conditions and biotic interactions that occur prior to and after disturbance (Decocq et al. 2004). Agroforestry practices have been shown to influence ECM fungal species richness and evenness (Hagerman et al. 1999; Byrd et al. 2000; Avis et al. 2003; Jones et al. 2003; Smith et al. 2005), being positively correlated with ecological mechanisms that re-establish plant variability, productivity, and biodiversity (Simard and Durall 2004). Hagerman and Durall (2004) reported that conservation of refuge host species following clear cutting practices increased the opportunity of out-planted seedlings to become colonized by a more diverse ECM fungal community. We did not test whether shrub strata contributed to ECM fungal diversity below-ground by establishing multiple mycelial networks with cork oak. However, it is well known that in ecosystems where multi-host ECM fungal species dominate, the percentage of ECM taxa shared can be high (Horton and Bruns 1998; Kennedy et al. 2003). Therefore, preserving patches of native vegetation in *montados* during cutting practices may be an important strategy to maintain the ECM fungal community (Dickie et al. 2004), conserve soil macrofauna (Sousa et al. 2004; Da Silva et al. 2008), aid the establishment of rare plants (Lavergne et al. 2005), and protect specific habitat of dependent small mammals and birds (Stoate et al. 2000; Klaa et al. 2005).

ECM fungal richness and diversity was lower in the DA-*montado* that was not managed for the last 15 years and is now a shrubland-like area with shrubs 2 m high occupying ca. 95% of total vegetation cover (Table 2). The transition of savannah-like landscape to *montados* dominated by shrubs may enhance the competition between trees and shrubs for nutrient resources and also increase the risk of large fires because large areas of shrubs are more ignitable than mosaics of natural pastures and annual crops (Nunes et al. 2005). This condition may cause long-term negative effects on vegetation resilience (Díaz-Delgado et al. 2002) and nutrient cycling (Carreira et al. 1997). Shrub management options, including the transition to shrubland-like landscape, and broader implications on the ECM fungal community resilience should be thoroughly studied in oak woodlands.

Concerning ECM fungal abundance, there was a positive correlation with agro-silvo-pastoral exploitation and recent soil tillage (Fig. 4). Traditional rotation cultivation in *montados* comprises periods with low vegetation cover at ground level due to crop production and/or shrub cutting practices. Our findings revealed that the maintenance of one to two vegetation strata, composed by shrubs and/or herbaceous plants (natural pastures and agricultural fields), did not affect ECM fungal abundance and apparently contributes to turnover of ECM fungal species. This niche separation in *montado* ecosystems, as consequence of management following a 9-year rotation cultivation system (Pinto Correia 1993b), also diminishes the light as a limiting factor (Moreno et al. 2005) and contributes for the maintenance of vegetation cover diversity (Decocq et al. 2004). In the context of ecosystem productivity, rooting patterns in such exploitation regime ensures the rapid turnover of the fine root system and creates a crucial pathway of carbon and nutrient flow from plant to soil (Baddeley and Watson 2005).

In the present study, by morphotyping ECM supplemented with ITS rDNA analysis and examining results in relation to *montado* landscape characteristics and land use practices, we were able to describe the ECM fungal community structure during the critical summer period. Since *C. geophilum* and families *Russulaceae* and *Thelephoraceae* represented 56% of the ECM fungal community, we can assume that these fungal symbionts play important roles in ecosystem functioning under drought conditions. The relationships established between ECM fungal richness and diversity regarding the *montado* landscape characteristics and land use practices provided an ideal opportunity to examine the influence of distinct explanatory variables on the ECM fungal community. In fact, although we did not directly address the ECM fungal community in regard to heavy oak mortality, we noticed that stands with absent/low cork oak mortality exhibited higher ECM fungal richness and diversity (Table 2; Fig. 2): FMa,b-*montado*, AMa,b-*montado*, OGa,b-*montado*. Oak woodlands are extremely susceptible to degradation and the situation becomes increasingly important under the scenario of global warming. The Iberian Peninsula has registered extremely warm years during the last decades, and it is already influenced by an increasing trend in temperature and decreasing precipitation (European Environment 2004). Climatic change will certainly have impacts on ecosystem functioning and may favor the establishment/aggressiveness of forest pathogens such as *Phytophthora cinnamomi*, strongly implied in cork oak mortality (Brasier and Scott 2008). Thus, it is important to continue characterizing the ECM fungal community in order to better understand the role of fungal symbionts as mediators between soil processes and plant community and to guide future management options to prevent cork oak mortality and sustain ecosystem productivity.

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Appendix

A1. Questionnaire used in the interviews to the farmers, land users and/or stakeholders

1. Identification of the responsible for the land exploitation
name/ age/ sex/ graduation/ profession

2. Identification of the type of land exploitation
name of property/ location/ total area/ date of beginning of land exploitation

2.1. Type of exploitation: ☐ familiar ☐ company
2.2. Acquisition of the property: ☐ inheritance ☐ purchase ☐ rented ☐ other; which?
2.3. House in the property: yes/no
2.4. Relation with property: ☐ lives in the property ☐ visits occasionally ☐ visits sporadically
2.5. Employees: y/n; if yes, ☐ permanently ☐ temporarily

3. Characterization of the *montado* landscape
3.1. Total area:
3.2. Savanna-like landscape: yes/no
3.3. Savanna-like landscape composed by:
 ☐ cork oak (*Quercus suber*) ☐ cork oak mixed with other species;
 which species?
 ☐ holm (*Quercus rotundifolia*); total area
 ☐ pinus (*Pinus pinaster*); total area
 ☐ pinus (*Pinus pinea*); total area
 ☐ other; total area

3.4. Shrub strata: density/ species present
3.5. Herbaceous strata: density/ species present
3.6. Cork oak mortality: ☐ absent ☐ present;
 if present, number of dead trees per hectare (ha):

3.7. Cork oak regeneration
 ☐ absent
 ☐ low (< 50 seedlings per ha)
 ☐ medium (50 to 100 seedlings per ha)
 ☐ high (> 100 seedlings per ha)

4. Land use in the *montado* landscape
4.1. Land use regime:
 ☐ none ☐ extensive ☐ intensive
4.2. Type of exploitation:
 ☐ silvopastoral; total area
 ☐ agro-silvo-pastoral, under a traditional 9-years rotation cultures system regime; total area
 ☐ agriculture; periodicity; total area
4.3. Soil exploitation:
 ☐ none; total area
 ☐ natural pastures; total area
 ☐ crop production, under a traditional 9-years rotation cultures system regime; total area
 ☐ crop production; periodicity; total area
4.4. Livestock:
 ☐ none ☐ extensive ☐ intensive
 if yes:
 ☐ cattle
 ☐ sheep
 ☐ iberian pig
 ☐ black goat
 ☐ other; which?
4.5. Mushrooms sampling: yes/no

5. Management planning

5.1. Cork harvesting: ☐ each 9-years ☐ other periodicity

5.2. Shrub management (density control):

☐ none
☐ by permanent grazing
☐ cutting practices with no soil tillage
☐ cutting practices followed by soil tillage
☐ other method; which?

5.3. Last shrub control (years):

5.4. Last soil tillage (years):

5.5. Nutrient supply: yes/no
if yes, last fertilizer input (years):

6. Technical support

6.1. Technical support: yes/no
if yes, in which?

☐ cork harvesting
☐ livestock
☐ shrub management
☐ soil analysis
☐ other; which?

7. Cork quality

7.1. Analysis related to cork quality: yes/no
if yes, which periodicity?

7.2. Cork quality

☐ low
☐ regular
☐ good

Table 3 Ectomycorrhizal material

Fungus symbiont	Voucher	ECM/reference/GenBank acc. number (*new)
Amanita rubescens	AAM 150/00, leg. 20.06.2000, VCb	*Quercirhiza internangularis*; Azul et al. 2001d; AJ889923*
Amanita sp2	AAM 156/00, leg. 20.07.2000, VCb	*Quercirhiza bruneoarenosa*; Azul 2002
Boletaceae sp1	AAM 121/00, leg. 20.07.2000, AMa	*Quercirhiza boletonivescens*; Azul 2002
Boletaceae sp2	AAM 199/00, leg. 20.06.2000, VCa	*Quercirhiza lecciummorpha*; Azul 2002
Cenococcum geophilum Fr.	AAM 103/00-II, leg. 20.06.2000, LTa	*Cenococcum geophilum* Fr., Azul 2002
Cortinariaceae sp1	AAM 179/00, leg. 20.06.2000, OGb	*Quercirhiza cortinarioglutinosa*; Azul 2002
Cortinariaceae sp2	AAM 195/00, leg. 20.06.2000, ESa	*Quercirhiza cortinariolanata*; Azul 2002
Cortinariaceae sp3	AAM176/00-I, leg. 20.07.2000, FMa	*Quercirhiza argenteoalba*; Azul 2002
Cortinariaceae sp4	AAM 140/00-I, leg. 20.06.2000, ESb	*Quercirhiza argenteoinflata*; Azul 2002
Cortinariaceae sp5	AAM 102/00-II, leg. 20.06.2000, OGa	*Quercirhiza argenteozonata*; Azul 2002
Genea sp1	AAM 133/00-II, leg. 20.06.2000, ESa	*Quercirhiza geneanitida*; Azul 2002
Lactarius chrysorrheus (Fr.) Fr.	AAM 117/00-II, leg. 20.06.2000, LTa	*Lactarius chrysorrheus* (Fr.) Fr.; Azul 2002; AF096983*
Lactarius sp1	AAM 118/00-II, leg. 20.06.2000, CÇ	*Quercirhiza lactofragilis*; Azul 2002
Lactarius sp2	AAM 186/00, leg. 20.06.2000, OGa	*Quercirhiza lactoflavescens*; Azul 2002
Lactarius sp3	AAM 194/00, leg. 20.06.2000, ESb	*Quercirhiza lactonitida*; Azul 2002
Pisolithus tinctorius (Pers.) Coker e Couch.	AAM 100/00-II, leg. 20.07.2000, SB	*Pisolithus tinctorius* (Pers.) Coker e Couch.; Azul 2002
Russula sp1	AAM 221/00, leg. 20.06.2000, CÇ	*Quercirhiza glutoepidermata*; Azul 2002
Russula sp2	AAM 171/00, leg. 20.07.2000, FMa	*Quercirhiza russuloacystidiata*; Azul 2002
Russula sp4	AAM 160/00, leg. 20.07.2000, FMa	*Quercirhiza russulongocystidiata*; Azul 2002

Table 3 (continued)

Fungus symbiont	Voucher	ECM/reference/GenBank acc. number (*new)
Russula sp5	AAM 141/00-II, leg. 20.06.2000, LTa	*Quercirhiza russulodecantata*; Azul 2002
Russula sp6	AAM143/00, leg. 20.06.2000, OGa	*Quercirhiza russuloepidermata*; Azul 2002
Russula sp7	AAM 225/00, leg. 20.06.2000, LTb	*Quercirhiza russuloflamescens*; Azul 2002
Russula sp8	AAM 190/00, leg. 20.06.2000, OGa	*Quercirhiza russulopallida*; Azul 2002; AF230897*
Russula sp9	AAM 196/00, leg. 20.06.2000, ESa	*Quercirhiza russulospinosa*; Azul 2002
Russula sp10	AAM 149/00, leg. 20.07.2000, AMa	*Quercirhiza russulosplendida*; Azul 2002
Russula sp11	AAM 180/00, leg. 20.06.2000, OGb	*Quercirhiza russulovillosa*; Azul 2002; AF418615*
Russula sp12	AAM 203/00, leg. 20.07.2000, DR	*Quercirhiza hirtuososplendida*; Azul 2002
Russula sp13	AAM 187/00, leg. 20.06.2000, OGb	*Quercirhiza melleacolor*; Azul 2002
Scleroderma areolatum Ehrenb.	AAM 101/00-II, leg. 20.06.2000, LTa	*Scleroderma areolatum* Ehrenb.; Azul 2002
Tomentella galzinii Bourdout	AAM 262/00-I, leg. 20.07.2000, SB	*Tomentella galzinii* Bourdout; Azul 2002
Tomentella sp1	AAM 164/00-II, leg. 20.07.2000, AMb	*Quercirhiza ateracusrugosa*; Azul et al. 2006a
Tomentella sp2	AAM 226/00-I, leg. 20.06.2000, LTa	*Quercirhiza auratercystidiata*; Azul et al. 2006b; AJ972618
Tomentella sp3	AAM 114/00-I, leg. 20.07.2000, AMa	*Quercirhiza summacornuta*; Azul 2002
Tomentella sp4	AAM161/00-II, leg. 20.07.2000, FMa	*Quercirhiza summatriangularis*; Azul et al. 2006e
Tomentella sp5	AAM 134/00-II, leg. 20.07.2000, FMa	*Quercirhiza tomentellocumulata*; Azul et al. 2008a; AM924140
Tomentella sp6	AAM 138/00-II, leg. 20.06.2000, ESb	*Quercirhiza tomentellocystidiata*; Azul et al. 2006f
Tomentella sp7	AAM129/00-II, leg. 20.06.2000, ESa	*Quercirhiza tomentelloflexuosa*; Azul et al. 2006g
Tomentella sp8	AAM 168/00-II, leg. 20.07.2000, FMb	*Quercirhiza tomentellofuniculosa*; Azul et al. 2006h; AM000027
Tomentella sp9	AAM167/00, leg. 20.06.2000, CÇ	*Quercirhiza tomentellomorpha*; Azul 2002
Tomentella sp10	AAM 162/00-II, leg. 20.07.2000, AMa	*Quercirhiza flavocystidiata*; Azul et al. 2006c; AJ972894
Tomentella sp11	AAM 208/00, leg. 20.07.2000, FMb	*Quercirhiza lanatriangularis*; Azul 2006d
Tomentella sp12	AAM 172/00, leg. 20.06.2000, VCa	*Quercirhiza brunneocolor*; Azul 2002
Tomentella sp13	AAM 124/00, leg. 20.06.2000, VCb	*Quercirhiza subcastanea*; Azul 2002
Tomentella sp14	AAM 157/00, leg. 20.06.2000, CÇ	*Quercirhiza brunneonuda*; Azul 2002
Tuber borchii Vitt.	AAM 139/00-II, leg. 20.06.2000, LTa	*Tuber borchii* Vitt.; Azul 2002; AF106890*
Unknown1	AAM 175/00, leg. 20.07.2000, FMa	*Quercirhiza aeneusocolor*; Azul 2002
Unknown2	AAM 184/00, leg. 20.06.2000, OGb	*Quercirhiza bicolornuda*; Azul 2002
Unknown3	AAM 166/00, leg. 20.06.2000, AMb	*Quercirhiza viridiocolor*; Azul 2002
Unknown4	AAM 217/00, leg. 20.07.2000, LTb	*Quercirhiza brunneovinosa*; Azul 2002
Unknown5	AAM 132/00, leg. 20.07.2000, ESb	*Quercirhiza mucoinflata*; Azul 2002
Unknown6	AAM 173/00-II, leg. 20.07.2000, FMa	*Quercirhiza sinuosopallida*; Azul 2002
Unknown7	AAM 151/00-II, leg. 20.06.2000, LTb	*Quercirhiza spongioflavens*; Azul 2002
Unknown8	AAM 181/00-II, leg. 20.06.2000, OGb	*Quercirhiza subrubescens*; Azul 2002
Unknown9	AAM 185/00, leg. 20.07.2000, OGb	*Quercirhiza tomentosoalba*; Azul 2002
Xerocomus subtomentosus (L.: Fr.) Quél.	AAM 158/00-II, leg. 20.06.2000, VCa	*Xerocomus subtomentosus* (L.: Fr.) Quél.; Azul 2002

The isolated ECM are deposited in the Centre for Functional Ecology, Department of Botany, University of Coimbra (COI), Portugal

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