Hinc patriam sustinet
Instituto Superior de Agronomia
Universidade Técnica de Lisboa

Influence of soil parameters on ectomycorrhizal diversity in *montado* ecosystems

Maria João Hortas Barrento

Dissertação para obtenção do Grau de Mestre em

Engenharia Florestal e dos Recursos Naturais

Orientador: Ana Paula Ferreira Ramos
Co-Orientador: Alberto Macedo Azevedo Gomes
Co-Orientador: Maria Helena Neves Machado

Júri:

Presidente: Doutor Manuel Armando Valeriano Madeira, Professor Catedrático do Instituto Superior de Agronomia da Universidade Técnica de Lisboa

Vogais: Doutora Maria Helena Reis de Noronha Ribeiro de Almeida, Professora Associada do Instituto Superior de Agronomia da Universidade Técnica de Lisboa

Doutora Celeste Maria Martins Santos e Silva, Professora Auxiliar da Universidade de Évora

Doutora Ana Paula Ferreira Ramos, Professora Auxiliar do Instituto Superior de Agronomia da Universidade Técnica de Lisboa

Licenciado Alberto Macedo de Azevedo Gomes, Investigador Auxiliar do Instituto Nacional de Investigação Agrária e Veterinária I.P.

Lisboa, 2012

Agradecimentos

À Professora Doutora Ana Paula Ramos pela orientação clara e entusiasta no decorrer deste trabalho.

Ao Investigador Auxiliar do INIAV Alberto Azevedo Gomes pela orientação neste trabalho, em especial pelo conhecimento que me transmitiu sobre o nosso *montado* e também pela descrição dos perfis do solo.

À Doutora Helena Machado, pela sugestão e orientação deste trabalho, em especial por me ter mostrado a grande diversidade de cogumelos e pela sua identificação.

À Professora Doutora Manuela Neves pela sua disponibilidade e simpatia na ajuda da análise estatística dos dados.

A todos os colegas envolvidos no projeto que permitiu a realização deste trabalho, em especial ao coordenador Doutor Edmundo de Sousa, ao Engenheiro António Saraiva e ao Doutor Abel Rodrigues pela colaboração no trabalho de campo. À Doutora Irene Cadima, pela realização do mapa da região de estudo, ao Doutor Jorge Capelo pelas explicações de estatística.

Ao Adérito Matos, Francisco Martins, Engenheiro João Soveral e Engenheiro Hernani Sobral também pela colaboração no trabalho de campo, ao Joaquim Martins pela colaboração na preparação das amostras de solo

Aos proprietários das herdades onde ocorreu a realização do trabalho, em especial ao proprietário Luís Dias.

À Clara Pinto e Márcia Silva pelo apoio que me deram ao longo do trabalho, à Teresa Sampaio por estar sempre disponível para ajudar, principalmente na estatística.

Ao meu pai, Faty, Manel e avó Irene por tornarem mais fácil este trabalho, à minha irmã São pelas correções do inglês.

Ao Paulo e às minhas filhotas Joana e Inês pela ajuda tão grande!

Abstract

This study characterized the composition and the diversity of macrofungal communities associated with four plots of *montado* situated in Grândola Hills, Southern Portugal, and evaluated the influence of soil parameters on ectomycorrhizal fungal diversity and abundance. Phosphorus, potassium, total nitrogen, organic matter concentrations and soil pH were the parameters determined. Differences in soil chemical features were found between studied plots. A total of 132 species of macrofungi were found in the study area being *Laccaria*, *Russula* and *Cortinarius* the most abundant genera. Also dissimilarities on macrofungal communities, particularly, on abundance and diversity, were registered among plots.

The influence of soil parameters on ectomycorrhizal diversity and abundance was studied in the plots with values of biological spectrum higher than one, and in particular for the most frequent species *Laccaria laccata*, *Cortinarius trivialis*, *Russula amoenolens* and *Russula subfoetens.* Results showed that ectomycorrhizal diversity is negatively correlated with the increasing of extractable phosphorus concentration, and the abundance of ectomycorrhizal species responds differently to soil chemical characteristics.

The present study allows us to understanding the influence of some soil features on ectomycorrhizal fungal diversity and abundance. Finally, the effects of management practices on ectomycorrhizal fungal diversity in this Mediterranean ecosystem are discussed.

Keywords: *Quercus suber*, macrofungi community, abundance, diversity indices, biological spectrum, shrub control

A influência de parâmetros do solo na diversidade ectomicorrízica nos ecossistemas montado

Resumo

Este trabalho pretendeu caraterizar as comunidades macrofúngicas associadas ao montado de sobro no Sul de Portugal quanto à composição e diversidade, e estudar a influência de parâmetros do solo na diversidade e abundância de fungos ectomicorrízicos. Determinaram-se os teores de fósforo, potássio, azoto total e de matéria orgânica bem como o valor do pH do solo, tendo-se encontrado diferenças nas características do solo entre as parcelas de estudo. Foram registadas 132 espécies de macrofungos na área de estudo, sendo *Laccaria*, *Russula* e *Cortinarius* os géneros mais abundantes.

A influência das características químicas do solo na diversidade e abundância de espécies ectomicorrízicas foi estudada nas parcelas onde se observaram valores superiores a 1 para o espetro biológico, e em particular para as espécies mais frequentes *Laccaria laccata*, *Cortinarius trivialis*, *Russula amoenolens* e *Russula subfoetens.* Os resultados mostram que a diversidade ectomicorrízica está relacionada negativamente com o teor de fósforo extratável e que a abundância das espécies ectomicorrízicas respondem diferentemente às caraterísticas edáficas.

Este estudo permitiu compreender a influência de algumas propriedades químicas do solo na diversidade e abundância dos fungos ectomicorrízicos. Deste modo, sugere-se a adoção de práticas de gestão que protejam a diversidade destes fungos neste ecossistema mediterrâneo.

Palavras-passe: *Quercus suber*, comunidade macrofúngica, abundância, indices de diversidade, espetro biológico, controlo de matos

Resumo alargado

Este trabalho teve como objetivo a caraterização das comunidades macrofúngicas, quanto à composição e diversidade, bem como o estudo da relação entre a diversidade e os parâmetros do solo em quatro montado de sobro situados na Serra de Grândola, no Sul de Portugal. Pretendeu-se ainda estudar a influência das caraterísicas químicas do solo na abundância de fungos ectomicorrízicos, em particular, nas espécies encontradas com maior frequência.

Em cada montado delimitou-se uma parcela quadrada, com uma área de 1 ha, subdividida em quatro sub-parcelas. Os carpóforos foram inventariados em todas as sub-parcelas durante a estação outonal, com cadência semanal, sempre que as condições climáticas o permitiram, e identificados até à espécie ou género. Para a sua identificação recorreu-se fundamentalmente a características morfológicas.

Em cada parcela caracterizou-se o tipo de solo de acordo com WRB (2006), através da descrição de um perfil de solo, e recolheram-se quatro amostras de solo da camada superficial em cada sub-parcela, cada uma constituída por 5 sub-amostras de 25 cm de profundidade. As análises foram realizadas no ex–LQARS e visaram a determinação dos seguintes parâmetros do solo: pH (H_2O), azoto total (N total), matéria orgânica (M.O.), fósforo extratável (P) e potássio extratável (K).

Para a caraterização das comunidades macrofúngicas procedeu-se à avaliação da abundância e diversidade. No que respeita à avaliação da abundância, estudou-se a possibilidade desta ser realizada a partir da contagem do indivíduo – micélio, por forma a tornar mais expedita a realização do inventário micológico. Assim, cada parcela foi caraterizada em termos de abundância de indivíduos e de diversidade, para a qual se determinou a riqueza específica (S) e os índices de Simpson (D) e de Shannon-Wiener (H'), e o espetro biológico. Para uma melhor avaliação das diferenças existentes na composição da comunidade macrofúngica entre as parcelas de estudo foi determinado o índice de similaridade de Jaccard (I_j).

Estudou-se a relação entre a diversidade dos fungos ectomicorrízicos e os parâmetros de solo avaliados, apenas em três das parcelas de estudo onde foram observados valores superiores a 1 para o espetro biológico, através de análise de regressão.

A relação entre os parâmetros do solo e a abundância total dos fungos ectomicorrízicos e das espécies mais frequentes foi também estudada através de análise de regressão.

Em toda a área de estudo foram registadas 132 espécies de macrofungos, das quais 107 são espécies ectomicorrízicas. *Laccaria*, *Russula* e *Cortinarius* foram os géneros mais abundantes, sendo *Laccaria laccata* a espécie mais frequente.

Em termos da composição da comunidade macrofúngica, a parcela Tanganhal Novo distingue-se fortemente das restantes por apresentar grande predominância de espécies saprófitas. No que diz respeito a abundância e diversidade de macrofungos bem como de fungos ectomicorrízicos, são as parcelas Barradas da Serra e Mostardeira que registam valores mais elevados.

Os resultados mostram que a diversidade de fungos ectomicorrízicos está inversamente relacionada com o teor de fósforo extratável, sendo este nutriente também o que explica melhor a variabilidade da abundância destes fungos. Assim, verificou-se um decréscimo da abundância dos fungos ectomicorrízicos para níveis mais elevados de fósforo. Este estudo ainda mostrou que a abundância das espécies mais frequentes responde diferentemente aos parâmetros do solo. *Laccaria laccata* e *Cortinarius trivialis* não mostram associação com nenhum dos parâmetros do solo estudados, apresentando um comportamento aparentemente ubíquo. Pelo contrário, as espécies de *Russula* estudadas, muito próximas taxonomicamente, apresentam comportamentos distintos. Enquanto *Russula amoenolens* parece estar associada a locais com solos mais ácidos, *Russula subfoetens* encontra-se possivelmente associada a condições de solo pobres em matéria orgânica, teores de fósforo e azoto total.

Este estudo permitiu ainda compreender que diferentes técnicas de controlo de matos podem influenciar a fertilidade do solo e consequentemente modificar a diversidade e abundância dos fungos ectomicorrízicos. Deste modo, sugere-se a adoção de práticas de gestão que protejam a diversidade destes fungos tão importantes para a sustentabilidade deste ecossistema mediterrâneo.

Contents

List of Figures

List of Tables

1. Introduction

1.1. The *Montado* ecosystem

Montado[1] is an agro-silvo-pastoral system (Pinto-Correia and Mascarenhas, 1999) resulting from the interaction of a long history of anthropogenic disturbances (Castro *et al*., 2010) which have influenced the landscape for many centuries in the Mediterranean Basin (Joffre *et al*., 1999). This system can be characterized by an open formation like savannah - type, mainly dominated by Mediterranean evergreen oaks, cork-oak (*Quercus suber* L.) and holm-oak (*Quercus rotundifolia* Lam.), taking place in monospecies or mixed stands (Pinto-Correia and Mascarenhas, 1999), with density ranging from 60 – 100 trees per ha (Barrico *et al*., 2010), combined with shrubs or rotation of crops, fallow and pastures as ground cover, where cattle, sheep, pigs and goats are extensively raised (Joffre *et al.*, 1988; San Miguel, 1994; Martín-Vicente and Fernández-Alés, 2006). This Mediterranean woodland can be composed to a lesser extent by the deciduous oaks, pyrenean oak (*Quercus pyrenaica* Willd.) and portuguese oak (*Quercus faginea* Lam.) (Joffre *et al.*, 1999), where olive tree (*Olea europaea* L.) and/or maritime pine (*Pinus pinaster* Aiton) and stone pine (*Pinus pinea* L.) can also be found (Bugalho *et al*., 2011).

Cork-oak ecosystems are widespread in the Western Mediterranean basin and cover about 2.5 million ha in Europe (Portugal, Spain, France and Italy) and North Africa (Morocco, Algeria and Tunisia). They cover about 800,000 ha in Portugal, which represent the largest area of distribution; Spain has also a good representation, while France and Italy contribute with a very low percentage. In North Africa, Morocco is the country with more distribution of this ecosystem (AFN, 2010; Pausas *et al.*, 2009).

This western Mediterranean evergreen oak tree belonging to Fagaceae occurs from sea level to an altitude of 500 m in hot and humid climates, in particular in the southern region of Iberian Peninsula influenced by the Atlantic Ocean and dominates and covers about 600,000 ha in the Alentejo region (AFN, 2010). *Quercus suber* usually grows in acidic soils on granite, schist, or sandy substrates (Pausas *et al*., 2009), but it can also occur, more rarely, on decalcified soils developed on carbonate rock (Montoya, 1980; Ruiz de la Torre, 2001; Sánchez-Palomares, 2007). Leptosols, Regosols, Cambisols and Luvisols are the main soil types associated with cork-oak stands (WRB, 2006).

[1] Like dehesa in Spanish

Montado are human-shaped ecosystems with high socioeconomic and conservation value (Bugalho *et al*., 2011) that have been managed for centuries supporting biodiversity (Plieninger and Wildbrand, 2001), being protected ecosystems under the Pan-European network of protected areas (Bugalho *et al*., 2011).

Their high level of structural diversity can be divided into two scales - on an intra-habitat scale, where the presence of scattered trees and their effects on the herbaceous layer cause habitat heterogeneity (Marañón, 1986) and into an inter-habitat scale, where the variety of types of *montado*, due to a variety of grazing, shrubs and cultures, and differences in composition, density and structure, enriches biodiversity (Díaz *et al.*, 1997). Besides, extensive cultivation within these systems increases habitat diversity and creates additional niches (Plieninger and Wildbrand, 2001).

Since the middle of the 20th century the land use has changed as far as undercover vegetation are concerned (Azul, 2011), as a consequence of the process of transformation of the agricultural sector in Europe, which threatened these ecosystems by poor or even non-existent land-management practices (Lourenço *et al*., 1998). This situation together with the decline of cork-oak, that have been especially evident during the two last decades in Portugal, are contributing to the vulnerability of this species (Azul, 2011). In fact, Pinto-Correia (1993) refers intensification and extensification as vulnerable situations.

It is necessary to counteract this vulnerability and promote their sustainability through the conservation of the *montado* biodiversity (Azul, 2011). According to Bugalho *et al*. (2011), the existence of *montado* ecosystems requires active management and man use.

This ecosystem provide habitat for several critically endangered species as the Iberian imperial eagle (*Aquila adalberti* C.L. Brehm), the Eurasian black vulture (*Aegypius monachus* L.), and the Iberian lynx (*Lynx pardinus* Temminck), among others (Carrete and Donázar, 2005). These ecosystems are also crucial for overwintering bird populations such as those of Eurasian cranes (*Grus grus* L.) and woodpigeons (*Columba palumbus* L.). As far as plants are concerned, more than 235 species of vascular plants can be found in an area of 0.1 ha of these ecosystems (Díaz-Villa *et al*., 2003), including a large diversity of shrub species (Bugalho *et al*., 2011).

Besides all diversity of animal and plant species, both highly diverse ectomycorrhizal (ECM) and saprotrophic fungal communities are associated with Mediterranean oaks (Richard *et al.*, 2004; Smith *et al.*, 2007). Generally, oak species are as rich in mycorrhizal as saprotrophic species than conifers (Moreau *et al*., 2002). In particular, results of a study concerning the presence of mycorrhizas in *montado* showed that the composition and structure of mycorrhizal fungi communities associated to cork-oak are much diversified (Azul, 2002).

According to Azul *et al*. (2011), 57 taxa of ECM fungi belonging to 17 genera have been assessed in a *montado* located in Montemor-o-Novo in Portugal during a 2-year sampling period. In other study conducted in *montado* stands located also in Alentejo region, 50 ECM fungal taxa were discriminated (Azul *et al*., 2010).

When macrofungal diversity of holm-oak and cork-oak woodlands was compared, the diversity in cork-oak stands seems to be higher than in the first one. For example, in a study conducted in Santarém district in Portugal, the number of genera distinguished in the site dominated by cork-oak was slightly higher than in the site dominated by holm-oak (Santos-Silva *et al*., 2011). Also, in a similar study conducted in Andalusia, southern Spain, with the particularity of over 50% of the data have been collected over the last 30 years and the rest compiled from the Andalusian mycobiota literature, 838 species were recorded, of which 78.8% belong to cork-oak woodlands (Ortega and Lorite, 2007).

A number of genera of Basidiomycota known to include species able to establish mycorrhiza with Fagaceae, such as *Amanita*, *Clitocybe*, *Cortinarius*, *Lactarius*, *Russula* and *Tomentella*, were referred in some studies as the most frequent genera found in the Portuguese cork-oak *montado.*

1.2. Mycorrhizal symbiosis

The organisms found in forest soils, which are mainly found below the surface of the litter layer, are crucial to the suitable functioning of forest ecosystems (Fisher and Binkley, 2000). Among Bacteria, Fungi and Algae, in terms of biomass, the Fungi generally dominate the soil microbiota (Killham, 1994).

All fungi are heterotrophs, requiring preformed organic compounds as sources of energy and carbon (Fisher and Binkley, 2000) and, depending upon the mode of nutrition, they can be divided into three major groups: saprophytes, which play an important role in forest soils increasing its fertility, decomposing proteins, cellulose, other carbohydrates and lignin (Wilde, 1958), parasites which are of great importance in causing diseases of plants (Pelczar *et al*., 1986) and symbionts or mycorrhizal fungi involved in the most frequent fungal infections within Kingdom *Plantae* (Martins, 2004). Although generally the mycorrhizal symbioses are considered mutualistic, due to the benefits for both the fungi and the host plant, recently the relation between the individual plant and the fungal symbionts is placed somewhere in the mutualistic-parasitic continuum, depending on their developmental state, the specific genotype combinations and the environmental conditions (Edmonds *et al.*, 2011).

Mycorrhizal fungi belong to Ascomycota, Basidiomycota and Zygomycota taxas (Martins, 2004) and form a symbiotic association with roots (mycorrhiza), which are involved in the nutrient uptake from soil (Smith and Read, 2008), while the host transfers to the fungus the organic carbon obtained by photosynthesis (Azul *et al*., 2008). Nutrients have necessarily to pass through a layer of fungus involving roots before being taken up by the phytobiont (Harley and Smith, 1983).

The association of fungi with roots of higher plants is very important to the nutrition and growth of trees and, therefore, in maintaining healthy trees in forests. Without mycorrhizae most of our most important trees could not survive long against the dynamic and competitive biological communities that inhabit forest soils (Fisher and Binkley, 2000).

Mycorrhizal condition is the normal state for the majority of the plants under most ecological conditions (Smith and Read, 2008). As an example, all the studied *Quercus* species depend on ectomycorrhizal fungi for their normal growth and survival under natural conditions (Barrico *et al*., 2010). Not all plants, however, have mycorrhizas.

Mycorrhizal symbiosis also plays a determinant role on ecosystem functioning, with benefits to plants such as plant growth in low nutrient availability, increasing uptake of phosphorus and nitrogen, drought tolerance, tolerance to heavy metals and other toxic compounds, and protection against pathogens (Azul *et al*., 2008, Barrico *et al*., 2010), and contributing to organic matter turnover and nutrient cycling (Fisher and Binkley, 2000).

According to Smith and Read (2008), seven mycorrhizal types can be considered: Arbuscular mycorrhiza, that are the most common mycorrhizal type, Ectomycorrhiza, Ectendomycorrhiza, Arbutoid mycorrhiza, Monotropoid mycorrhiza, Ericoid mycorrhiza and Orchid mycorrhiza.

Ectomycorrhizas (ECM) are the dominant forms in most forest trees (Marks and Foster, 1973) and are characterized by the presence of three structural components: a mantle of fungal tissue enclosing the tips of fine roots, the Hartig net, a labyrinthine hyphae system growing between the epidermal and cortical cells, and external mycelium, which form essential connections with the soil and the sporocarps of the fungi forming ectomycorrhizas.

Most of ECM species are Basidiomycetes, belonging to large families as Amanitaceae, Boletaceae and Russulaceae (Martins, 2004), but some Ascomycetes and some Zygomycetes (within the genus *Endogone*) can also form ECM.

Ectomycorrhizae are associated with the roots of most extratropical trees belonging to Betulaceae, Corylaceae, Fagaceae, Myrtaceae, Pinaceae, Salicaceae and giant tropical Asian trees of the Dipterocarpaceae (Smith and Read, 2008).

Contrary to obligatory parasitic fungi which present high host specificity, mycorrhizal fungi in general present low specificity. Despite this lower specificity, some specificity at genera level has been described and each host species tends to have characteristic groups of fungi

capable of establishing mycorrhizae (Fisher and Binkley, 2000). Within the ECM fungi most species provide an association with various plant hosts (Martins, 2004) and many different Basidiomycetes are able to form association with the same tree species simultaneously (Fisher and Binkley, 2000).

The presence and the composition of mycorrhizal communities, and particularly those of ECM, can be considered as bioindicators in studies concerning the impact of human disturbances on forests, like global change, the effects of pollution or forest management practices due to their crucial position at the plant-soil interface.

Among the most important factors that affect the density and species diversity of soil organisms, are the supplies of oxygen and moisture, soil temperature, levels of inorganic nutrients, and the amount and character of soil organic matter (Fisher and Binkley, 2000). While activities of many common bacteria, algae and actinomycetes are inhibited by highly acidic conditions, fungi are able to grow and reproduce over a wide range of pH; thus, microbial population of acid forest soils are dominated by fungi as a consequence of the lack of competition for food supply (Fisher and Binkley, 2000).

Susceptibility of the root to mycorrhizal infection appears to be influenced by photosynthetic potential and soil fertility (Marx, 1977), where low to moderate soil fertility enhances mycorrhizal development, while the opposite conditions may reduce or even prevent it (Fisher and Binkley, 2000). Particularly in Vesicular Arbuscular Mycorrhizas, the development of the association between the host and mycobionts is often strongly suppressed by increasing of available phosphorus and nitrogen.

Mycorrhizal development, besides the strong influence of available concentrations of soil nutrients, is controlled by soil pH, which changes the bioavailability of both nutrients and toxins, and by soil water, being generally inhibited by both excessively low and high moisture regimes. Excessively high moisture is inhibitory because all soil fungi are aerobes and low soil moisture reduces mycorrhizal development as a consequence of water stress on both mycobiont and phytobiont, or of changes in nutrient availability (Killhan, 1994).

Also, soluble carbohydrates of roots must be considered a major factor in the formation of ectomycorrhizae, the growth of vegetative hyphae, and the development of reproductive structures. Thus, factors that change the photosynthetic activity, availability of root sugars and soil nutrients are of the major importance to carbohydrate metabolism of associated fungi (Hacskaylo, 1973).

Beyond forest composition, structure and age, mycorrhizal communities seem to be highly shaped by soil nutrients (Santos-Silva *et al.,* 2011), in particular, the nature and dynamics of soil organic matter and phosphorus contribute to its composition and distribution (Barrico *et al*., 2010). In addition, Kranabetter (2009) refers that one fundamental aspect of

ectomycorrhizal fungi ecology is the relationship between soil nitrogen availability and ECM species distribution and diversity.

1.3. Assessment of macrofungal abundance and diversity

Macrofungal abundance can be accessed through fruiting bodies quantifying or by evaluation of soil mycelium using molecular tools (Barrico *et al.*, 2010).

Due to ephemeral nature of fungal fruiting bodies, to get a good estimation of species richness, based on sporocarps inventory, it is necessary to collect several times during the fruiting season and during multiple years. However, some uncertainty is often associated with fungal diversity studies (Schmit *et al*., 1999). In the case of mycorrhizal fungi, it was referred that sporomes have been considered a poor measure of the mycorrhizal status of the tree roots (Gardes and Bruns 1996; Richard *et al.*, 2005; Smith *et al.*, 2007). In fact Gardes and Bruns (1996) showed that, in general, mycorrhizal mycelia found on the roots of trees correspond poorly with the fruiting bodies which are above. Although studies on fungal species diversity are increasingly performed with molecular techniques (Landeweert, 2005), fruiting bodies assessment have the advantage of providing information about fungal characteristics and distribution patterns (Peter *et al.*, 2001a; Baptista *et al*., 2010).

In order to evaluate species diversity, the most used parameters are the ones relative to diversity within a community (α diversity), represented by the number of species present into a homogeneous particular community (Moreno, 2001). There are methods based only on species number quantification as species richness, which is one of the oldest and most fundamental used in community ecology, and others based on community structure, that is, based on proportional abundance. Proportional or relative abundance indices indicate the level of equitability (evenness) or dominance, being some of the most known based on the evenness concept (Moreno, 2001). Evenness indices express the uniformity and assume that individuals are randomly selected and all species are included in the sample, while dominance indices are inverse parameters to the equitability concept, as they only take into account the representatives of species with higher importance value, not considering the contribution of the ones with lower importance.

The Shannon-Wiener evenness index is an example of evenness indices commonly used at macrofungal diversity studies, which ranges from zero, when only one species is present (low equitatibility), to logarithm S (ln S), when all species share the same representativeness (Magurran, 1988). As dominance index, the Simpson´s index is also much utilized. It ranges from 0 to 1. When the value is near 0 signify high species diversity, that is, any species present dominance.

Species diversity can also be evaluated through the assessment of diversity between habitats (β diversity), which is the level of differences on species composition between communities (Moreno, 2001). For this purpose the Jaccard similarity index can be utilized. It ranges from 0, when there's any species in common for both places, to 1 when they have the same species composition.

Despite all the variety of indices for diversity assessment, the choice should take into account those mostly used in similar studies and the fact that using different parameters to analyse the same data could origin redundant results (Moreno, 2001).

Biological spectrum, consisting in the quotient between ectomycorrhizal and saprotrophic species, can also be useful to compare the mode of life of the species present within a plot according to Moreau *et al*. (2002).

The main goal of this thesis, linked to "Study and demonstration of management practices for cork-oak *montado* recuperation" project, funded by the "Fundo Florestal Permanente", in co-operation with the INIAV (Instituto Nacional de Investigação Agrária e Veterinária), CAP (Confederação dos Agricultores de Portugal) and AFN (Autoridade Florestal Nacional) was to characterize the macrofungal diversity in cork-oak ecosystems in Portugal and evaluate the effect of some nutrients and soil chemical features, like pH, on ECM fungal diversity and abundance.

Four stands of cork-oak, located at Grândola Hills, in the southern part of the country were selected by differentiating themselves in soil characteristics, topography, land uses and shrub management practices.

With this thesis we hope to contribute to a better knowledge on the macrofungal diversity associated with *Q. suber*, promoting biodiversity conservation of the *montado*.

Additionally, the results will also improve the rural development in the *montado* ecosystem by means of understanding the best soil conditions that promote ectomycorrhizal diversity, contributing to the mitigation of the decline of cork-oak stands in the Mediterranean area.

2. Material and methods

2.1. Stand characteristics

The study was performed in Grândola Hills (38º6'N-8º63'W; 325 m above sea level), located in the Alentejo region, southern Portugal (Fig. 1).

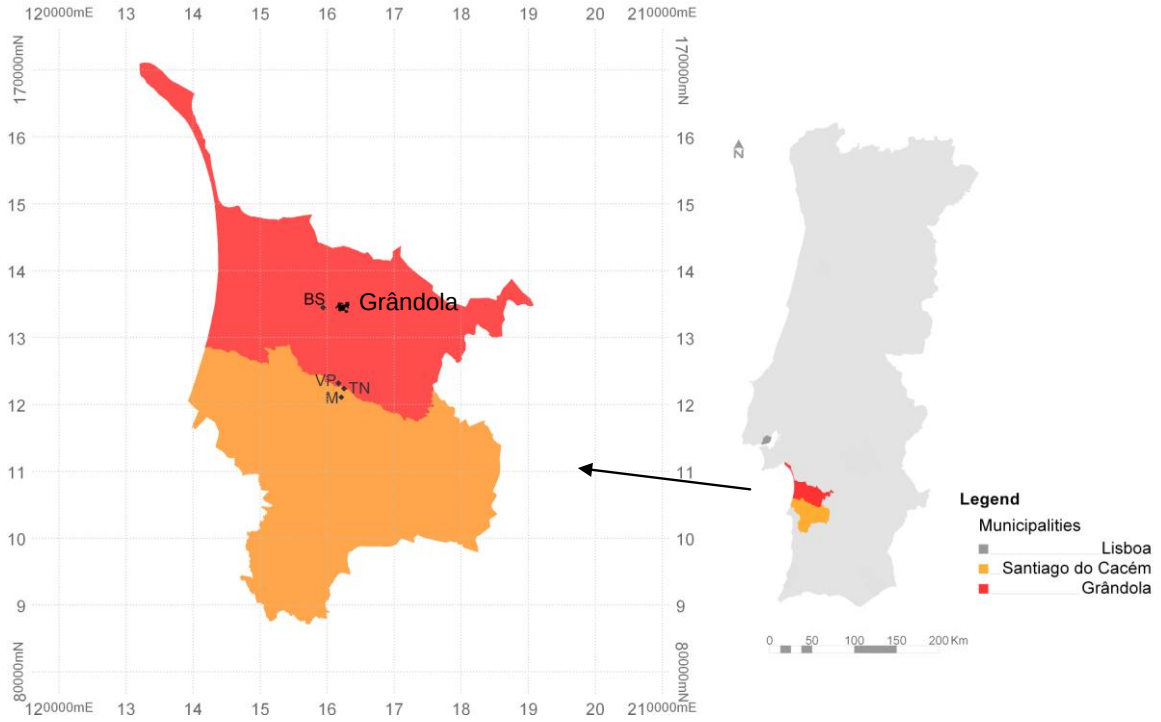


Figure 1 - Location of the stands where the studies were performed: Grândola and Santiago do Cacém municipalities, in Southern Portugal (BS - Barradas da Serra; M – Mostardeira; TN - Tanganhal Novo; VP - Várzea dos Pereiros) (cordially made by Irene Cadima).

The climate in this region is typically Mediterranean, characterized by hot dry summers (Buscardo *et al*., 2010) and mild winters, with some Atlantic influence. The annual rainfall is 676 mm and the average annual temperature is 15.6 ºC (INMG, 1991).

In the study area geological formation is dominated by schist-greywacke complex with vertical stratification (Inverno *et al*., 1993).

Grândola Hills is covered mainly by cork-oak *montado* and by little areas with different occupation of land such as groves, riparian galleries and mixed oak of *Q. suber* and *Q*. *faginea*. In *montado* areas develops a rich Mediterranean shrubland composed mainly by

Cistus spp. (rockrose (*Cistus ladanifer* L.) and Montpelier cistus (*Cistus monspeliensis* L.)), in which often occur rosemary (*Rosmanirus officinalis* L. and heathers (*Erica* sp.) (Lourenço *et al*., 1998; Rebelo, 2009).

Four square plots of 1 ha, each corresponding to different local land use situations and traditional management practices in the region, were selected (Fig. 2): Barradas da Serra (BS) located in Grândola municipality, Mostardeira (M), Tanganhal Novo (TN) and Várzea dos Pereiros (VP) located in Santiago do Cacém municipality. The plots were divided into four subplots with 0.25 ha each one and all trees within the plots were previously numbered. Each subplot within the plot was assumed independent from the neighbouring ones. All selected plots are monospecific areas of *Q. suber*, except BS, which also includes *P. pinea* (Fig. 2).

Figure 2 - Aspects of the four plots sampled: BS - Barradas da Serra; M - Mostardeira; TN - Tanganhal Novo; VP - Várzea dos Pereiros (cordially photographed by Helena Machado).

Different land use and shrub management practices have been applied in the previous years in the studied areas. BS, M and TN have a forest use with occasional ovine grazing, whereas VP is characterized by a silvo-pastoral system with ovine grazing. Fertilization was used at BS and TN. The last time fertilization occurred in BS was in the year 2006, whereas in TN it took place in 2007 when yellow lupine seeding occurred.

At BS shrub density is controlled with shredder cuts every 4 years, having the last intervention taken place in the year of the present study, 2010. At VP the shrub density is controlled by means of disc harrow every 6 years, having the last one taken place in 2005. At TN it is done by shredder cuts and sometimes by disc harrow, every 4 years and the last one occurred in 2007. Contrary to the other situations previously presented, at M the shrub control has been made by hand and by occasional grazing. The general characteristics of the sampling plots are shown in Table 1.

Table 1 - Localization and general characteristics of the sampling plots. Geographic coordinates, altitude, geology, soil type, relief, exposure, land use, shrub control practices and density (trees/ha)

	BS	**M**	**TN**	**VP**
Geographic coordinates	38º11'03''N 8º37'14''W	38º03'22''N 8º33'49''W	38º04'06''N 8º33'30''W	38º04'34''N 8º34'02''W
Altitude (m)	215	208	225	225
Geology	shists	sandstone upon shists	shists	shists
Soil type	Leptosols	Cambisols	Cambisols	Cambisols
Relief	steep slope	planed slope	modereted slope	modereted slope
Exposure	North	_	South	West/south-west
Land use	forest	forest	forest	silvopastoral
Shrub control practices	shredders cuts	manual control	shredders cuts/disc harrow	disc harrow
N (trees/ha)	210	147	135	137

BS - Barradas da Serra; M – Mostardeira; TN - Tanganhal Novo; VP - Várzea dos Pereiros.

2.2. Soil sampling and analysis

Soil sampling was performed in all plots from December 2010 to March 2011.

In order to classify the soil type, for each plot a 110 cm depth hole was opened to observe soil profiles (Fig. 3) and horizon description. Soils were classified according to WRB (2006).

Figure 3 - Soil profiles of the plots: BS - Barradas da Serra; M – Mostardeira; TN - Tanganhal Novo; VP - Várzea dos Pereiros (cordially photographed by Azevedo Gomes).

Four subplots were delimitated in each study area. Four samples, resulting from five cores, with 25 cm depth, were taken in each subplot and stored in plastic bags identified and transported to laboratory where they were air dried (Fig. 4 A).

All samples were sifted through a sieve of 2 mm (Fig. 4 B) to separate fine from coarse particles, stored in plastic bottles and then transported to the ex - Rebelo da Silva Agricultural Chemical Laboratory, where analyses were performed.

Figure 4 - Preparation of soil samplings.

Standard soil parameters, pH (H_2O), total nitrogen (N), organic matter (OM), extractable phosphorus (P), extractable potassium (K) were measured, according to the following methods (LQARS, 2006):

i) pH(H_2O) was determined by potenciometry method in a soil:water suspension in the ratio 1:2,5 (v/v);

ii) Organic matter content was determined multiplying the organic carbon content by factor of 1.724 (LQARS, 2006), assuming that organic matter contains 58% of carbon. Organic carbon was determined by dry combustion, according to the ISO 10694 Norm;

iii) Total N was also determined by dry combustion, using an elemental analyzer LECO CNS, according to Norma ISO 13878;

iv) Extractable P and K were determined by the modified Egnér-Riehm method, using as extracting a solution of ammonium lactate 0,1 N and acetic acid 0,4 N at pH ranging from 3,65 to 3,75, being dosed by plasma emission spectrophotometry with a optical detector (ICP-OES simultaneous radial);

2.3. Fruiting body inventory and macrofungal identification

Fruiting body inventory was done in all sampling subplots/plots during the autumn season, once a week, whenever weather conditions allowed that, from 15th November to 10th December 2010. The sporocarps encountered in the sampling plots were counted and photographed. The sample number, the species name (when identified), and the number of closest tree were registered (see field data sheet – Annex 1). One or two specimen of each

species were collected, stored in paper bags identified with the sample number and transported to the laboratory to be identified.

The fruiting bodies collected were identified to species or subspecies level. The specimens that could not be identified to species level were classified by a numerical code.

Standard macroscopic characteristics, available literature (Basso, 1999; Munõz, 2005; Sarnari, 2005; Sarnari, 2007; Sánchez, 2008) and suitable field guides (Bon, 1988; Courtecuisse, 2000; Gerhardt, 2000; Lozano, 2001) were used to identify the samples.

Microscopic features (an Olympus BX41 microscope was used) and chemical reactions (potassium hydroxide or iron sulphate reactions) were also considered. The parameters considered, such as the shape and colour of spores, colour of the latex that flows after gills cut of *Lactarius* spp., potassium hydroxide or iron sulphate reactions to test colour changes to identify some species, are shown on Figure 5.

2.4. Macrofungal characterization

2.4.1. Macrofungal abundance

In this study, only the identified species and those which were assigned with a number were considered in macrofungal abundance analysis.

Macrofungal abundance was quantified using two basic methods. The first method, that has already been used in several studies, concerning macrofungal diversity, was based on the counting of the number of fruiting bodies that a species produces during the season period in a given area. The second method consisted in counting the trees where a given species can be found, that is, the frequency is calculated as the number of trees in which a species occurs. Thus, in the first method the individual is the sporocarp whereas in the second one, the individual is the mycelium associated to one tree, considering that the mycelium origins all fruiting bodies present in its canopy projection area. Therefore, it is assumed that fruiting bodies found in the same tree on multiple visits indicate the persistence of the same mycelium.

For each subplot and plot, the number of fruiting bodies (n1) and the number of individuals (n2) for each species were determined.

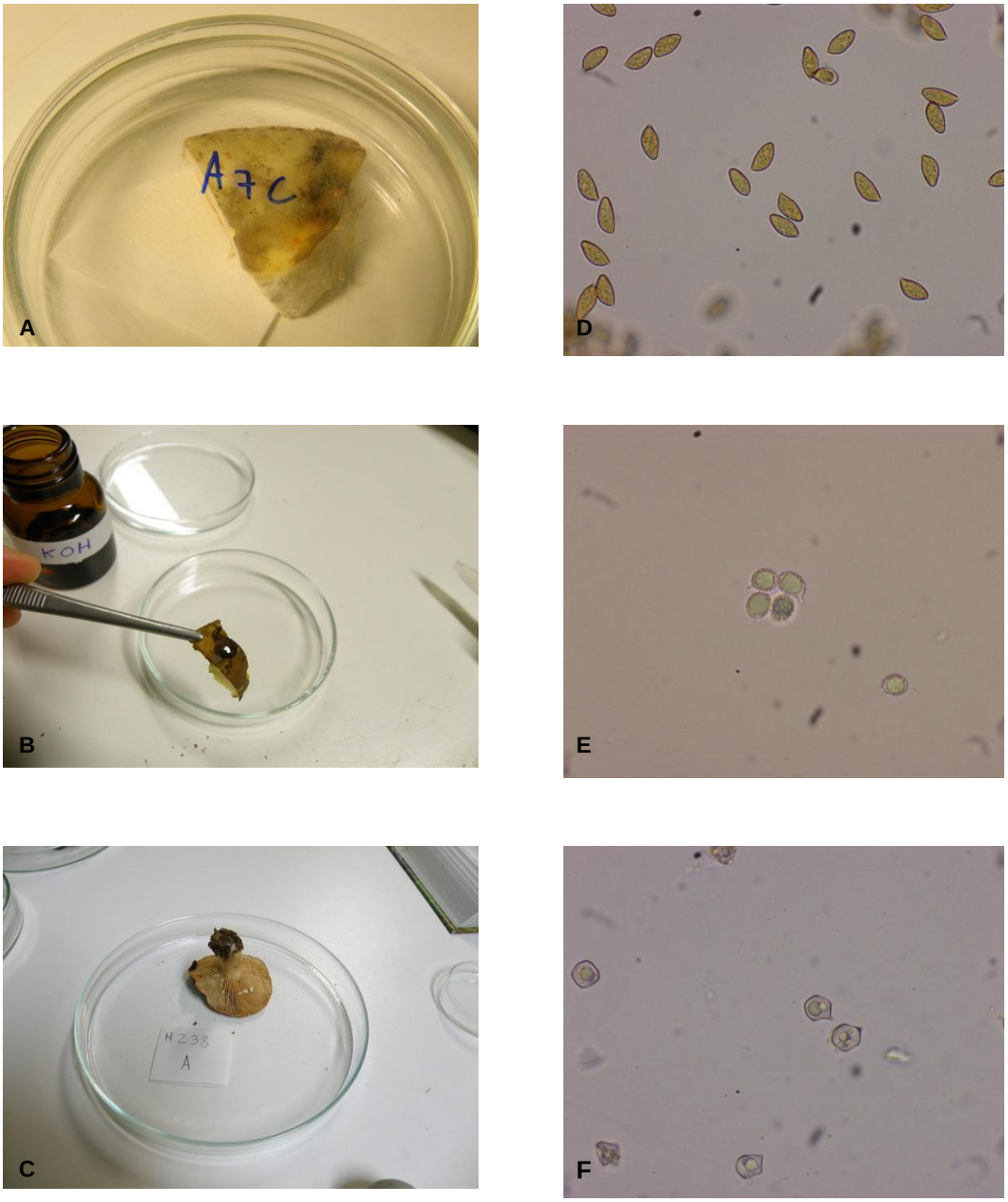


Figure 5 - Some of the morphological features used to identify species of macrofungi. A: colour of the spore print of the gills of *Russula* species; B: reaction to KOH to identify *Russula subfoetens*; C: latex production by *Lactarius* sp.; D: spores of *Cortinarius* sp.;E: spores of *Russula* sp.; F: spores of *Entoloma* sp.

2.4.2. Macrofungal diversity

Each macrofungal taxa was classified according to trophic category in saprotrophic, parasitic and mycorrhizal. For data analysis the only parasitic fungal species found was included within the saprotrophic group.

For each plot, macrofungal species richness, ectomycorrhizal species richness, saprotrophic species richness, Shannon-Wiener index (H') (evenness index) and Simpson index (D) (dominance index) were determined. Jaccard similarity index was also calculated to investigate differences in macrofungal composition between plots.
Only these indices were applied because, once using different parameters to analyse the same data, redundant results can be originated (Moreno, 2001).
The indices described below were calculated using the number of individuals, as follow:

Shannon-Wiener evenness index: H' = - $\sum$ **pi ln pi** (Moreno, 2001)
where **pi** is the relative abundance of species **I** (**pi** = **ni/N**) and **ni** is the number of individuals of species **I** and **N** is the total number of individuals;

Simpson´s index: D = $\sum$ $\mathbf{pi^2}$ (Moreno, 2001)
where **pi** is the relative abundance of species **i** (**pi** = **ni/N**), **ni** is the number of individuals of species **I** and **N** is the total number of individuals;

Jaccard similarity index: I_j **= c/(a+b-c)** (Moreno, 2001)
where **a** is the number of species presents on place **A**, **b** is the number of species presents in place **B** and **c** is the number of species presents in both places.

For each plot the biological spectrum was also calculated following an adaptation of the work of Moreau *et al*. (2002):
Biological spectrum = number of ECM species/number of saprotrophic species

2.4.3. Ectomycorrhizal diversity and abundance

The ectomycorrhizal diversity was evaluated only at BS, M and VP, where the ectomycorrhizal fungi were well represented (biological spectrum > 1).
For each plot/subplot species richness, Simpson (D) index (dominance index), Shannon-Wiener (H') index (evenness index) were determined as well the total number of individuals and the percentage of mycorrhized trees.

2.5. Statistical analysis

Non parametric Kruskall-Wallis and Mann-Whitney U-tests were used to test differences in soil parameters between sites because the normality of distribution and homogeneity of variance were not verified for all soil variables. The normality was tested through Shapiro-Wilk test and the homogeneity of variance by Levene Statistic. When significant differences were found through Kruskall-Wallis tests, Post-hoc paired differences were evaluated with Mann-Whitney *U*-tests (Annex 2).

In order to study the existence of relationship between the number of fruiting bodies and the number of individuals, a linear regression was adjusted between the Logarithm of both variables. The data were log transformed because the variable number of fruiting bodies did not present a normal distribution (Annex 3).

Associations between ectomycorrhizal fungal diversity and soil parameters were verified through regression analysis, after verifying the normality of diversity variables using Shapiro-Wilk tests (Annex 3).

There were adjusted regression models in order to determine the existence of correlations between soil parameters and abundance of total ectomycorrhizal species and of some particular species. When abundance variables did not present a normal distribution, data were Log transformed (Log (x +1)) (Annex 3).

Statistical analysis was performed using SPSS 20 software package (IBM SPSS Statistics version 20).

3. Results and discussion

3.1. Soil type and soil parameters

The soil description was based on the guidelines for soil description according FAO-ISRIC (1990), in which, there were distinguished the mineral surface horizon characterized by human intervention (Ap), the mineral subsurface horizon (B), the weathered horizon (C) and hard bedrock (R). There were also distinguished transitional horizons with properties of two horizons (RC, A/RC, B/C, R/C), vertical divisions (successive layers) within horizon C (C1 and C2), and a discontinuity also within horizon C (2C), that indicates a difference in the material from which the horizon is formed.

The main soil features observed on soil profile horizons (Fig. 6) for each plot are described as following:

Barradas da Serra

Ap (0 - 10 cm): Reddish brown (5YR $5/4_{(dry)}$), dark reddish brown 5YR $3/4_{(wet)}$); sandy loam texture with common rock fragments of shale stone (grit, fine and medium gravels and stones); fine and medium subangular blocky structure, common fine and very fine roots; friable, slightly hard consistence, sticky and plastic.

A/CR (10 - 25 cm): Light reddish brown (5YR $6/4_{(dry)}$), yellowish (5YR $5/6_{(wet)}$); loam texture with common rock fragments of schist stone (grit, fine and medium gravels and stones); medium angular blocky structure; few medium roots; slightly hard consistence, firm, sticky and plastic.

CR (25 - 110 cm): Compacted and continuous schist rock, presenting the schistosity plans vertical position, with development of roots in depth.

Mostardeira

Ap (0 - 15 cm): Pale brown (10YR $6/3_{(dry)}$), dark brown (10YR $4/3_{(wet)}$); sandy loam texture with few fragments of quartz (sand, fine and medium gravel and very few stones); fine and medium subangular blocky structure; many fine, very fine and medium roots; hard consistence, friable, slightly sticky and slightly plastic.

B_W/C (15 - 27 cm): Very pale brown (10YR $7/3_{(dry)}$), pale brown (10YR $6/3_{(wet)}$); loam texture with few fragments of quartz (sand, fine and medium gravel and very few stones); Very fine and medium subangular blocky structure; many fine, very fine and medium roots ; slightly hard to hard consistence, friable, slightly sticky and slightly plastic.

C1 (27 - 40 cm): Strongly weathered parent material with many fine roots and some thick roots.

C2 (40 - 60 cm): Weathered parent material with root development in depth.

2C (60 - 110 cm): Strongly weathered schist rock with roots growing in depth.

Tanganhal Novo

Ap (0 – 15 cm): Light brown 7.5YR $6/4_{(dry)}$, dark brown 7.5YR $4/4_{(wet)}$; sandy loam texture with many weathered rock fragments of schist stone (grit, fine and medium gravels and stones); fine subangular blocky structure; few fine and very fine roots;,slightly hard consistence, friable, slightly sticky and slightly plastic.

B_W/C (15 – 30 cm): Light brown 7.5YR $6/4_{(dry)}$, reddish brown 5YR $5/4_{(wet)}$; sandy clay texture with many weathered rock fragments of shale stone (grit, fine and medium

gravels and stones); medium angular blocky structure; very few medium roots; slightly hard consistence, friable, sticky and plastic.

C (30 – 55 cm): Continuous weathered and fractured schist rock, presenting the schistosity plans vertical position, with very few fine roots developing in depth.

CR (55 – 110 cm): Compacted schist rock with very few roots along the craks.

<u>Várzea dos Pereiros</u>

Ap (0 – 20 cm): Reddish brown (5YR 5/4$_{(dry)}$, dark reddish gray (5YR 4/2$_{(wet)}$; sandy loam texture with common rock fragments of schist stone (grit, fine and medium coarse gravels and stones); Fine and medium subangular blocky structure; fine, very fine and medium roots; slightly hard consistence, very friable, slightly sticky and slightly plastic.

C/B$_w$ (20 – 40 cm): Light brown 7.5YR 6/4$_{(dry)}$, reddish brown 5YR 4/4$_{(wet)}$; clay loam texture with common rock fragments of schist stone (grit, fine and medium coarse gravels and stones); medium subangular blocky structure; fine, very fine and medium roots; soft consistence, friable, sticky and plastic.

R (40 – 90 cm): Compact and continuous schist rock, with root development in depth, along the cracks.

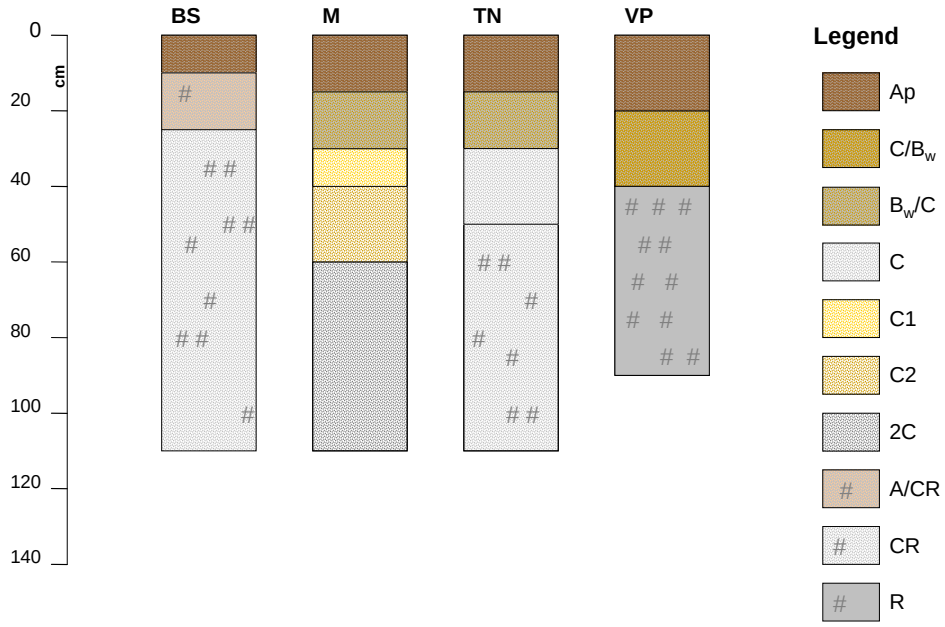


Figure 6 – Soil profiles of study plots according FAO-ISRIC (1990): BS - Barradas da Serra; M – Mostardeira; TN - Tanganhal Novo; VP - Várzea dos Pereiros.

BS is characterized by Leptosols soils while at M, TN and VP the soils are Cambisols. M has a different pedogenesis as a result of the colluvial deposition of latest soil materials over schist rock from the Carbonic, having the mineral fraction of these soils different features from those of the other plots.

These soils correspond to the two main types of soil association with cork-oak, Leptosols and Cambisols (WRB, 2006), and the profiles are in agreement with the characteristic soil type of the portuguese *montado* with profiles undeveloped and the rocky substrate situated near the surface (Natividade, 1990).

Significant differences were found in soil parameters between plots (P< 0.05) (Table 2).

Table 2 - Soil parameters measured at the four plots (average value ± SE, n=4)

Soil parameters	Sampling plots			
	BS	M	TN	VP
Ext P (mg kg^{-1})	9.92 ± 0.63^{b}	6.96 ± 0.62^{a}	13.58 ± 0.61^{c}	19.01 ± 0.93^{d}
Ext K (mg kg^{-1})	210.95 ± 6.53^{d}	60.87 ± 5.32^{a}	134.09 ± 9.91^{b}	104.43 ± 5.80^{c}
Total N (%)	0.13 ± 0.00^{b}	0.06 ± 0.00^{a}	0.13 ± 0.01^{b}	0.18 ± 0.01^{c}
O.M. (%)	3.11 ± 0.14^{b}	1.63 ± 0.01^{a}	2.90 ± 0.03^{b}	3.85 ± 0.30^{c}
pH (H_2O)	5.6 ± 0.08^{c}	5.2 ± 0.08^{a}	5.3 ± 0.06^{ab}	5.4 ± 0.04^{b}

Different letters after average value ± SE indicate significant differences (Mann-Whitney, $p < 0.05$) among columns (plots); BS - Barradas da Serra; M – Mostardeira; TN - Tanganhal Novo; VP - Várzea dos Pereiros.

Soil extractable phosphorus (P concentration) varied significantly between all sampling plots ($p<0.05$, for all Mann-Whitney tests between plots). The lowest value observed (6.96 mg kg^{-1}) was registered at M and the highest was at VP (19.01 mg kg^{-1}). Although significant differences were registered, the values observed for all plots are considered very low (<25 mg kg^{-1}) (Soveral-Dias *et al.,* 1980).

As with P concentration, all plots differed significantly from each other relative to extractable potassium concentration. This nutrient was significantly higher at BS (210.95 mg kg^{-1}) and significantly lower (60.87 mg kg^{-1}) at M than in the other plots. According to Soveral-Dias *et al*. (1980), the value registered at BS is very high (> 200 mg kg^{-1}), while the values registered at TN and VP were high (101 – 200 mg kg^{-1}) and at M were medium (51 – 100 mg kg^{-1}).

Total N concentration was significantly higher (0.18 %) at VP than in the other plots and was significantly lower at M (0.06 %). No significant differences were found between BS and TN

(U=7.00, p=0.77). Generally, the values found are higher when compared with the value obtained for soil sampling belonging to Ap horizon of Px profile in the Alentejo region, which was 0.07 % (Cardoso, 1965). It was also higher than that reported (0.10 %) for the *montado* located in the center of the Alentejo region (Herdade da Mitra) (Otieno *et al.*, 2011), except for M, where 0.06 % was registered.

Organic matter concentration (O.M.) was significantly higher (3.85 %) at VP and was significantly lower (1.63 %) at M than in the other plots, and not varied significantly between BS and TN (U = 5.00, p = 0.39). According to Costa (2004), while M presents a low level of O.M., the values registered in the other plots are considered as medium.

The soil at BS had a significantly higher pH (H_2O) (5.6) than the pH of the other three plots. The lowest value of pH was registered at M (5.2) although not significantly differing from that registered at TN (U = 4.00, p = 0.23). TN and VP soils did not differ on what concerns the pH value (U = 4.500, p = 0.294). According to Costa (2004), the soil at BS is considered slightly acidic while in the other plots soils are acid.

Most cork-oak woodlands occur on soils with pH generally ranging from 4.7 to 6.5 (Serrasolses, 2009), which is in accordance with the values found in all study plots. Values of pH in the range 3.6 - 6.5 and 3.9 - 5.3, were referred too, respectively to Leptosols and Cambisols soils for cork-oak forests and woodlands situated in Alentejo and in other regions of Portugal (Moreira and Martins, 2005).

In general, the soil chemical characterization shows that all study plots had very low level of extractable phosphorus, although significant differences were registered among them, BS differs mainly from others by the highest level of extractable potassium and because of slightly acidic soil, while M was the poorer plot relative to all nutrients. VP presented the highest concentrations of extractable P, total N and O.M., and TN, showed, in general, intermediate values for the considered soil parameters. Although this parameters varied between plots, the general characterization of the study area correspond to the soil conditions where cork-oak predominantly occur, that is soils with low P and N status, but generally rich in potassium (Natividade, 1990).

3.2. Macrofungal community characterization

3.2.1. Macrofungal abundance

A total of 3139 sporocarps and 706 individuals were assigned to 132 taxa (Annex 4). Of those, 2786 sporocarps and 629 individuals, belonging to 105 taxa, were sorted into ECM fungi.

The most abundant families were: Tricholomataceae with 10 taxa (38.52% of total fruiting bodies; 17.00% of total individuals), Russulaceae with 55 taxa (29.15% of total fruiting bodies; 39.24% of total individuals), and Cortinariaceae with 8 taxa (13.16% of total fruiting bodies; 12.61% of total individuals).

A total of 35 genus were identified, being the most representative *Laccaria* (33.74% of fruiting bodies; 13.60% of total individuals), *Russula* (23.80% of fruiting bodies; 33.14% of total individuals) and *Cortinarius* (13.16% of fruiting bodies; 12.61% of total individuals) (Fig. 7), although a considerable number of fruiting bodies identified belonged to *Amanita*, *Boletus*, *Clitocybe*, *Gymnopilus*, *Hygrophorus*, *Lactarius* and *Tricholoma* genera (Fig. 8).

In genus *Laccaria* the only species found was *Laccaria laccata* (Scop.: Fr.) Cooke, by far the most abundant species during the observations, although absent from the plot TN. Among *Russula* genus, *Russula amoenolens* Romagn. was the most predominant, with 5.22 % of total fruiting bodies and 4.96 % of total individuals (although being present only at M and VP plots) and within *Cortinarius* genus the most numerous species was *Cortinarius trivialis* J. E. Lange with 7.04 % of total fruiting bodies and 5.38% of total individuals (absent from TN).

Between plots differences on the composition of macrofungal species were observed. *Cortinarius* is the only genus that appears in all plots. On the contrary, there is some genera that appears only in one of the plots: *Armillaria*, *Hygrophorus*, *Hypholoma* and *Suillus* occurred only at BS, while *Auricularia*, *Helvella* and *Phallus* was observed only at VP; *Clitopilus*, *Collybia*, *Lycoperdon* and *Pisolithus* occurred only at M, and *Lepiota* and *Pluteus* appeared at TN. On the other hand *Amanita*, *Boletus*, *Hebeloma*, *Inocybe*, *Laccaria*, *Lactarius* and *Russula* did not appear at TN, while *Agaricus* was absent only from VP; finally, *Gymnopilus* and *Macrolepiota* were not present at BS.

Apparently, the occurrence of *Suillus* species only in BS is related with the presence of *P. pinea*. In fact the symbiotic association between this fungus and pine trees is very well documented and referred as very frequent in pine forests in Portugal (Pinho-Almeida and Baptista-Ferreira, 2005).

Although it is difficult to compare total macrofungal richness obtained with those achieved from other previous studies where inventory of fruiting bodies were usually done during more than one year and in areas of different size, the number of total macrofungal taxa found in this study seems to be in agreement with other reported by Azul *et al*. (2011) for cork-oak *montado* (114 taxa) and by Santos-Silva *et al*. (2011) for cork-oak and holm-oak (123 taxa). However, the total macrofungal richness found in the present study is higher when compared to the data referred by Barrico *et al*. (2010) obtained in the Sado-Ribatejo region, Central-West Portugal (48 different taxa for *montado* of *Q. suber*).

Relatively to the ratio between mycorrhizal and non mycorrhizal species, the value observed in the present study, indicates the predominance of ECM over saprotrophic species, which is

quite similar to the situation described by Barrico *et al*. (2010) but higher when compared with those mentioned by Azul *et al*. (2011) and Santos-Silva *et al*. (2011), where the saprobes dominated.

Almost all most abundant genera found in the present study were previously reported in cork-oak *montado* in Portugal. Santos (1948) referred the occurrence of *Boletus* sp., in particular *Boletus edulis* Bull., *Russula violacea*, *Russula delica*, *Russula lactea*, *Russula alutacea*, *Armillaria* and *Lactarius deliciosus* associated with cork-oak. These same genera were reported as associated with the cork-oak root system by Torres Juan (1975). More recently, according to Barrico *et al*. (2010), *Lactarius*, *Russula*, *Cortinarius* and *Clitocybe* were the genera with more fruiting bodies inventoried and *Laccaria laccata* was also present in a considerable number. Beyond *Russula* and *Lactarius*, Azul *et al*. (2011) referred also *Tomentella* and *Amanita* as the most abundant genera, and *Laccaria laccata* and *Astraeus hygrometricus* as the most abundant species.

The higher values of abundance were registered at M and BS plots. At M, 1546 sporocarps and 325 individuals were found while at BS 1155 sporocarpps and 281 individuals were registered. At VP the abundance was characterized by 332 sporocarps and 85 individuals, while TN by 106 sporocarps and 15 individuals.

Concerning particularly the ectomycorrhizal communities, BS is characterized by the presence of species belonging to different genera such as *Laccaria*, *Russula*, *Cortinarius*, *Lactarius*, *Amanita* and *Tricholoma*. On the contrary, plot M presents few genera than BS and is dominated by *Laccaria*, *Russula*, *Cortinarius* and *Lactarius*, while at VP, *Laccaria*, *Russula*, *Cortinarius* and *Scleroderma* are the dominant genera. One unique ECM species (*Cortinarius decipiens*) was found at TN.

The high occurrence of *Laccaria laccata* might be explained by the fairly abundant presence of *Cistus ladanifer* on all the plots, as this association has been well documented (*Torres et al*., 1995).

A

B

C

Figure 7 – Sporocarps of some of the most represented genera of macrofungi found: A - *Cortinarius trivialis*; B - *Laccaria laccata*; C - *Russula amoenolens*.

A

B

C

Figure 8 – Sporocarps of some genera with some representativeness: A - *Lactarius deliciosus*; B - *Boletus aereus*; C - *Amanita pantherina.*

In order to verify the possible relationship between the number of fruiting bodies (n1) and the number of individuals (n2), a linear regression was adjusted with the logarithm of each variable (Fig. 9). This analysis was performed using those species which did not present neither scarce nor too abundant fructification.

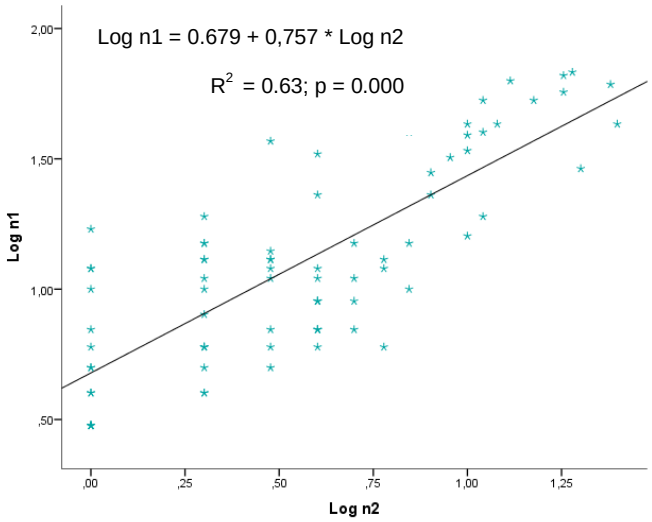


Figure 9 – Relationship between logarithm of number of fruiting bodies (Log (n1)) and logarithm of number of individuals (Log (n2)), n = 80.

Although regression was significant, the precision was weak (63 %), indicating very different fructification patterns among species. Thus, species which produce more sporocarps are overestimated on abundance evaluation using the method of fruiting bodies counting. Therefore, quantifying the number of individuals allows us to compare abundance of species ecologically very different (Schmit *et. al*., 1999), with the advantage of making the inventory more practical (no need of counting all sporocarps found). The establishment of this type of relationship for each species is of great importance, mainly in species with economic value, facilitating production evaluation.

All analyses which follow were performed using the number of individuals (n2) as unit for quantifying abundance.

3.2.2. Macrofungal diversity

The highest values for species richness were observed at M and BS, while intermediate values were found at VP; TN was the plot with the lowest values (Table 3).

The values of biological spectrum observed indicate that all plots, with the exception of TN, have predominance of mycorrhizal over saprotrophic species (the quotient is more than 1), being the predominance clearly evident at BS and M.

Through Simpson index (D) observation, the values near zero indicate higher diversity for all plots, and no species was considered as dominant.

Table 3 - Macrofungal diversity evaluated by species richness (S), Simpson index (D), Shannon–Wiener index (H') biological spectrum

Sampling plots	S	D	H'	Biological spectrum
BS	73	0.04	3.68	17.73
M	78	0.05	3.66	11.50
TN	13	0.08	2.52	0.07
VP	31	0.06	3.06	2.86

BS - Barradas da Serra; M – Mostardeira; TN - Tanganhal Novo; VP - Várzea dos Pereiros

Regarding the Shannon–Wiener index (H') for each plot and taking into account the superior limit (4.29, 4.36, 2.56 and 3.43, for BS, M, TN and VP respectively), the values relative to BS and M indicate high equitability between species and the values registered for TN and VP indicate very high equitability.

Jaccard similarity index (I_j) was determined to investigate differences on macrofungal composition among plots (Table 4). The value observed for BS versus M was 0.30 (the higher value registered) while for BS versus VP and for BS versus TN were, respectively, 0.08 and 0.01. This index for M versus VP and for M versus TN was also low, 0.16 and 0.06, respectively. Similarly, for VP versus TN the index value was 0.16. Therefore, according to the Jaccard similarity index BS and M are the plots that share more species (30%).

Table 4 - Jaccard similarity index (I_j) between plots

Comparison between plots	BS vs M	BS vs VP	BS vs TN	M vs VP	M vs TN	VP vs TN
Ij	0.30	0.08	0.01	0.16	0.06	0.16

BS - Barradas da Serra; M – Mostardeira; TN - Tanganhal Novo; VP - Várzea dos Pereiros

Thus, according to the results for all the indices used, plots BS and M present higher species richness than VP and TN, sharing more species than all the others. On the contrary, lower species richness with predominance of saprotrophic over ectomycorrhizal fungi separate TN from the other plots while VP represents an intermediate situation between these two distinct cases. All plots present high equitability and low dominance, consequently they present high diversity. However, from the results found the species richness is the best diversity descriptor to evaluate the real situation observed among plots.

Therefore, it is considered that BS and M have the highest macrofungal diversity being TN the poorest one. This last plot is characterized by a small number of adult trees while young trees (from basal sprout), dead jack stands, trunks and branches are abundant. The richness of decomposing plant litter over the soil is probably contributing to the predominance of saprotrophic fungi in this plot. On the other hand, environmental conditions created by the denser canopy cover present in the other plots improve mycorrhizal richness and reduced saprotrophic production (Santos-Silva *et al.*, 2011) which can explain the very unequal situation observed. Apparently, the steep decline of TN is reflected on the macrofungal composition, which is predominantly saprophyte, having been found only one ectomycorrhizal species (*Cortinarius decipiens*), as previously described.

Interestingly, these results might be, somehow, related with the shrub management carried out in each plot. In BS and M, where the highest macrofungal diversity was found, shrubs were controlled through shredders cuts and by hand, while in TN and VP the control was done by harrowing. According to Barrico *et al.* (2010), harrowing has an important negative impact on macrofungal richness and diversity which, in contrary, might be favoured by minimizing soil mobilization operations.

3.3. Diversity and abundance of ectomycorrhizal fungi related to soil parameters

The study of the relationships between ectomycorrhizal abundance and diversity and parameters was performed only at BS, M and VP plots, where the ectomycorrhizal fungi

were well represented. Table 5 shows the characterization of diversity and abundance of ECM species for these plots.

It is found that BS and M presents largest species richness than VP, and regarding to other diversity descriptors, all plots have higher diversity. Simpson index (D) values ranges from 0 to 1 and those close to 0 indicate high diversity. As can be seen by observing table 5, for all plots this index presents values very lower, thus the ectomycorrhizal diversity is higher for three plots. The same result is obtained through Shannon-Wiener index values. It ranges from 0 to 4.2, 4.2 and 3.0, respectively to BS, M and VP and for those the values are near the upper limit. Contrary to Simpson index values near 0 indicate lower diversity.

In conclusion, BS and M presents greater ECM abundance as well as higher ECM fungal diversity than VP, mainly when diversity is analyzed through species richness.

Table 5 – Ectomycorrhizal fungal diversity evaluated by species richness (S), Simpson index (D), Shannon–Wiener index (H'), and total number of individuals

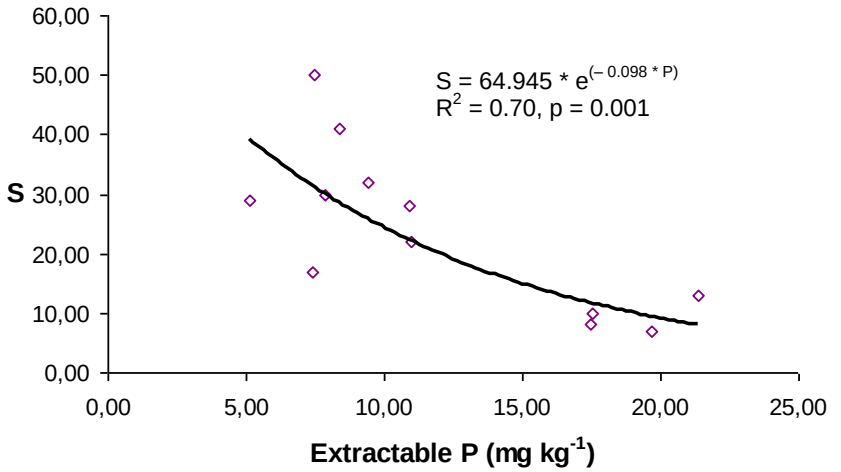
Sampling plots	S	D	H'	Total Individuals
BS	65	0.05	3.55	266
M	67	0.06	3.49	299
VP	21	0.09	2.70	63

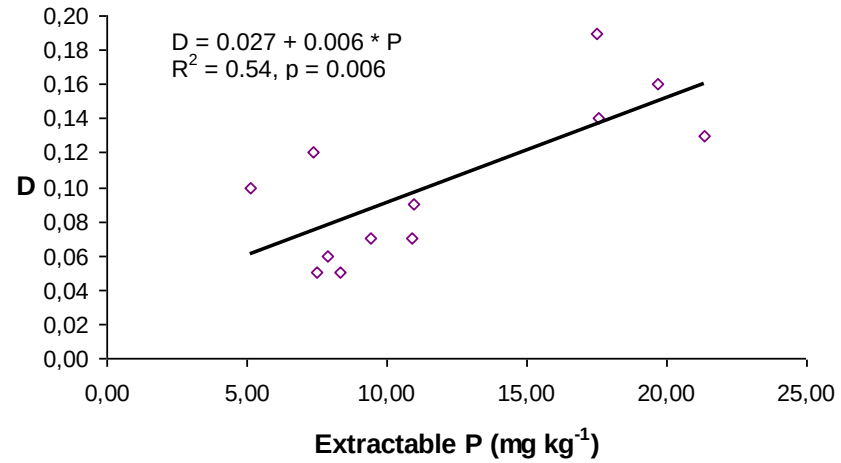
BS - Barradas da Serra; M – Mostardeira; VP - Várzea dos Pereiros

3.3.1. Relationships between ectomycorrhizal fungal diversity and soil parameters

For the purpose of investigating the existence of relationships between ectomycorrhizal fungal diversity and soil chemical properties, regressions were performed between S, D and H' variables and soil parameters (extractable phosphorus, extractable potassium, total N and organic matter contents and pH) determined for each subplot, in a total of twelve subplots.

There were only adjusted significant regression models between the diversity descriptors and the extractable soil P concentration (Fig. 10).





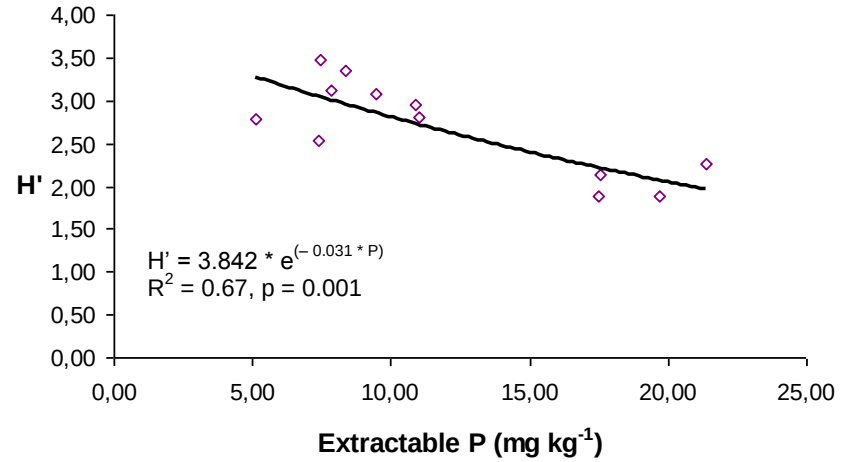


Figure 10 - Relationships between diversity descriptors (Species richness (S); Simpson index (D) and Shannon-Wiener index (H')) and extractable phosphorus.

Considering only the regression models adjusted with a reasonable accuracy (with S and H' indices), since the model adjusted with D and the P content has a precision of 54 %, species richness (S) and diversity of ECM fungi are negatively correlated with soil P content. That is, an increase in the P content might contribute to diminish species richness (S) and diversity of ECM fungi. Although the relationship between D and this nutrient was very low, also the trend shows the reducing of diversity with the increasing of extractable P (higher values of D indicate low diversity). Under the present study the ectomycorrhizal fungal diversity was not significantly associated with potassium, total N, organic matter contents neither with pH.

Taking into account the soil chemical characterization, the study plot with higher levels of P, showed less ECM richness and diversity, while BS and M with lower concentration of this soil parameter, presented high ECM richness and diversity. This result is according with Moreau *et al*. (2002), who referred that plots very rich in phosphorus presented a very low ECM diversity. A negative relationship between soil available P and ECM fungal richness was also reported by Twieg *et al*. (2009).

Although associations between diversity descriptors and the other soil parameters studied were not found, these have been referred by other authors. Reverchon *et al*. (2012) referred that species richness was significantly and negatively correlated with total C and N contents, indicating it was higher at the less fertile sites. According to Killham (1994), the development of the association between the host and the mycobiont, in particular to the Vesicular Arbuscular Mycorrhizas, is often suppressed by high levels of available phosphorus and nitrogen. The same author refers that soil pH is also a strong control of mycorrhizal development.

If the sample size had been larger, probably it would have been possible to obtain correlations with higher precision and correlations with other soil parameters that have been described as determinants to richness and diversity of ECM fungi.

VP was the plot where the levels of O.M., as well as P and total N, were significantly higher than at the other sites, which may be a consequence of the management practices used. In fact, this plot is characterized by a silvo-pastoral system with frequent ovine grazing and by periodic soil mobilization (harrowing for shrub control). Therefore, the inputs of O.M. are reflected in soil nutrient availability, in particular in P and N. In contrast, the lower level of these parameters was registered at M, which, despite having different pedogenesis, is subjected to occasional grazing and manual shrub control.

This seems to agree with Barrico *et al*. (2010), who stated that different land management practices cause changes in the nature and dynamics of soil organic matter and associated nutrients, including phosphorus.

Noting the association of ECM richness and diversity with less fertile sites, they are of major importance on forest soils, which are generally phosphate-deficient soils. Much of the

phosphate in soil is firmly bound to the soil complex of mineral and organic matter, so that plant roots can only absorb a small proportion of total phosphate available in the soil solution but when rootlets are infected, the mineral nutrients absorbing can be more efficient. It has been recognized that the most beneficial effect of mycorrhizas is the improvement of P nutrition of plants. They develop a wider physical exploration of the soil than roots and affects chemical changes and P solubility differently by roots which could lead to access to inorganic and organic P sources that are unavailable to non-mycorrhizal plants (Cardoso, 2006).
Increase in N absorption is also an important role of mycorrhizas, since this nutrient is limiting in forest production and mainly in acidic soils (Ana-Magán and Fernández, 2000). About 98 % of total N is connected to O.M. and its mineralization depends, among other factors of pH: when pH values range 6 – 8, the N availability is maximum, while out of this interval the availability of this nutrient decreases readily.
Furthermore, the results obtained conjugated with those reported by other authors underlines the importance of taking into account the type and levels of fertilizers used on *montado* management practices, in particular, relative to crops and pastures in ground cover. In conclusion, fertilization should not be used extensively without a previously evaluation of local fertility. For example, phosphate deficiency can be avoided by ensuring that suitable mycorrhizal fungi are present when young trees are planted (Garrett, 1981). For the same purpose, attempting to contribute to the ECM diversity, caution should be taken on the choice of shrub control technique, reducing the use of heavy machinery.

3.3.2. Relationships between ectomycorrhizal abundance and soil parameters

Association between ectomycorrhizal abundance and soil parameters were studied through the investigation of interactions between phosphorus, potassium, total nitrogen, organic matter contents and pH, and the percentage of trees mycorrhized per subplot as well as the percentage of trees per subplot with the presence of some particular ectomycorrhizal species like *Cortinarius trivialis*, *Laccaria laccata*, *Russula amoenolens* and *Russula subfoetens*. These species correspond to those most abundant within more representative genera and occurring at least at two plots (8 subplots). Regression models were adjusted to data in order to explain the abundance variability in function to soil variables referred above.

The regression models that were adjusted with a reasonable precision are presented in Fig. 11. There were adjusted regression models between the percentage of trees mycorrhized and extractable phosphorus, total N and O.M. concentrations, and the model

found with higher precision (78 %) was that between the percentage of mycorrhized trees and extractable phosphorus concentration.

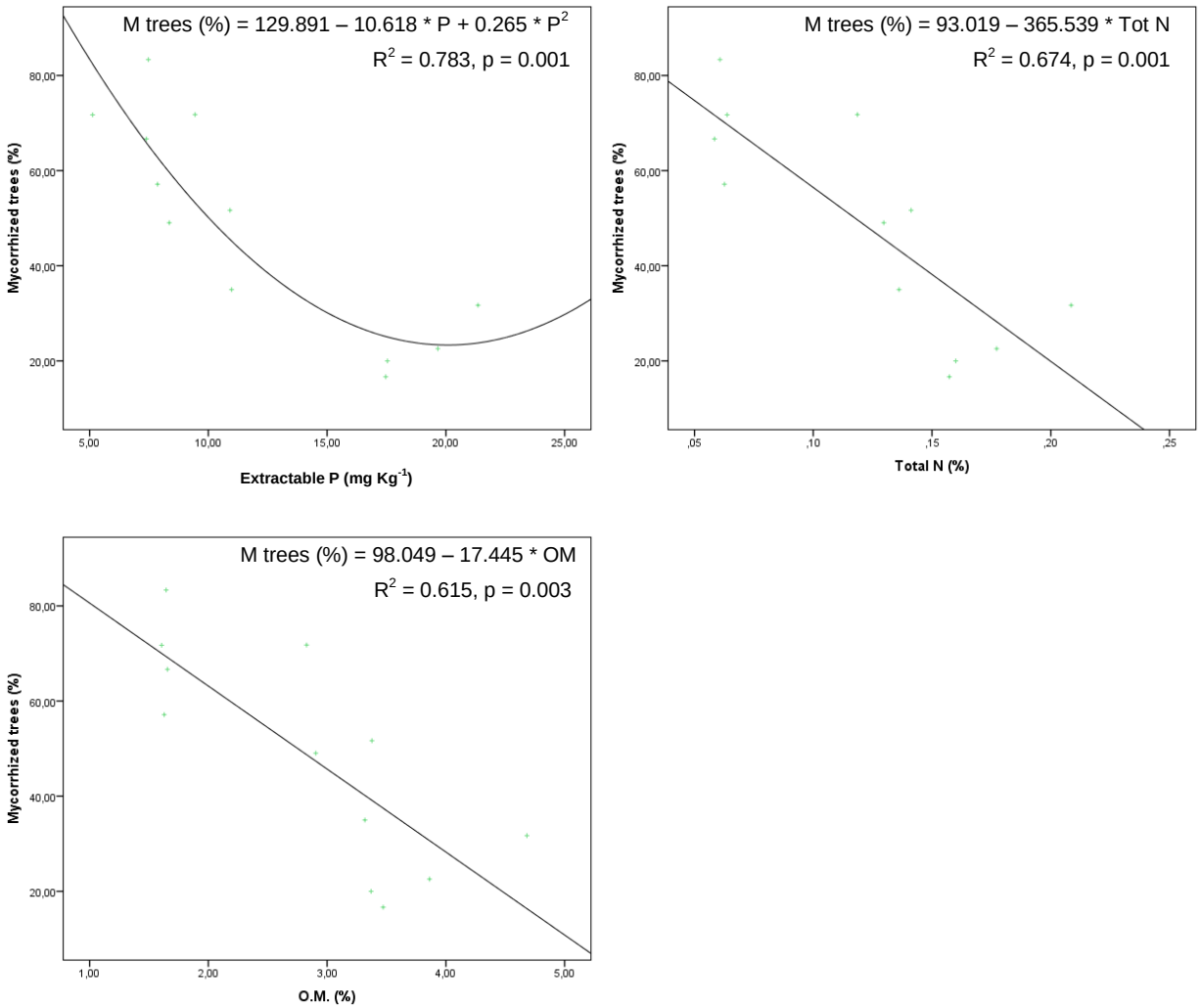


Figure 11 - Relationships between the percentage of mycorrhized trees and soil parameters: extractable P, total N and O.M. (n = 12).

The variability of percentage of mycorrhized trees is explained with 78 % of accuracy through the quadratic regression model. This percentage decreases until a level of about 20 mg kg^{-1}. Above this value the trend seems starting increase, however, the sample size does not allow a rigorous evaluation and the probably trend observed contradicts the fact that high soil fertility may reduce or prevent mycorrhizal development as was referred by Fisher and Binkley (2000). With lower accuracy, and explained through linear models, the abundance of ECM fungi decreases when total N and O.M. increase. Again, if the sample size had been larger, certainly the precision of the equations would have been improved.

The results show the possibility of ECM abundance being associated with poor soil conditions, mainly relative to phosphorus and total N contents. This in agreement with Reverchon (2012), that despite describing a study on *Pinus montezumae* Lam. in Mexico, reported that abundance of ECM sporomes were higher at less fertile sites. Likewise, it was suggested by Baar and ter Braak (1996) that small contents of nutrients and organic matter were favourable to ECM fungi.

A significant but not very high linear regression model was adjusted between the logarithm of percentage of trees with the presence of *Russula amoenolens* and soil pH (Fig. 12). The variability of the logarithm of trees with the presence of this species is explained only by 63 % of this model. Although the precision obtained, this result could suggest that this species inhabit more acidic environments.

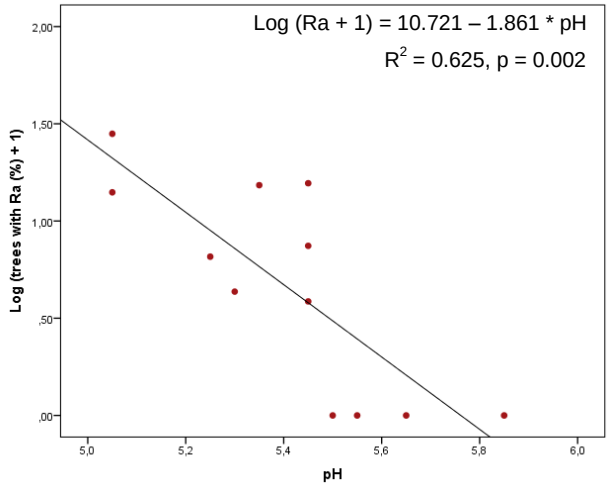


Figure 12 - Correlation between logarithm of percentage of trees with *Russula amoenolens* and soil pH (n =12).

This is in agreement with our previous observations as this species was mostly predominant at M where the soil pH was the lowest, whereas in the other plots (with higher pH values), its occurrence is lower and even null. According to Ortega and Lorite (2007), the acidity of the substrate influences highly the fructification of *Russula* spp. and *Lactarius* spp.

The significant quadratic models adjusted between the percentage of trees with *Russula subfoetens* and soil parameters, phosphorus, total N and O.M. contents are shown at Figure 13.

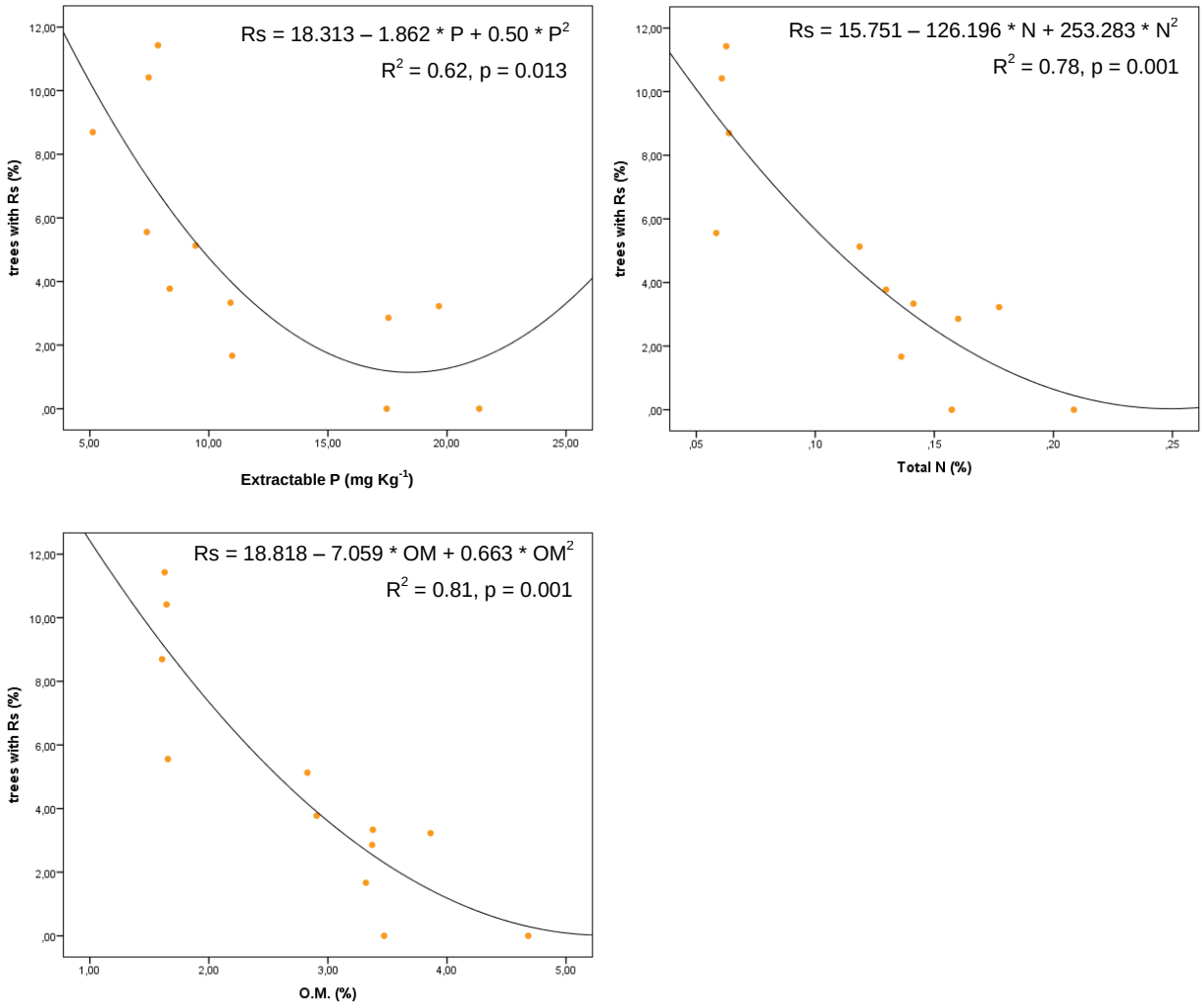


Figure 13 - Relationships between percentage of trees with *Russula subfoetens* and soil parameters: extractable P, total N and O.M. (n = 12).

The variability of the percentage of trees with *Russula subfoetens* is explained with a precision of 62 %, 78 % and 81 %, through the regressions resulting of the association with phosphorus, total N and O.M., respectively. This indicates possible negative correlations between the abundance of *Russula subfoetens* and P, total N and O.M. concentrations, although stronger with the last two ones. Regarding to extractable P, the regression shows that the abundance of this species decreases until a certain level (about 18 - 19 mg kg^{-1}) and after, there is a trend to increase. Similarly to the relation between the percentage of mycorrhized trees and this parameter, in this case it would be necessary a higher sample size to assess rigorously this association. These results do not allow concluding about the possible relationships between this macrofungus and P availability, but can permit to understand the possible influence that the concentrations of N and O.M. could have in the

abundance of this species. It seems that *Russula subfoetens* prefers sites poor in O.M. and N.

No association was found between the percentage of trees with the presence of *Laccaria laccata* and *Cortinarius trivialis* and soil variables studied in this analysis. Although this result, *Cortinarius trivialis* occurred mainly in BS and only one individual was found at VP, and the presence of *Laccaria laccata* was predominant at M.

Nevertheless, the absence of correlations between abundance of these species and soil characteristics, suggests that these species could inhabit in different environmental conditions, presenting ubiquity on what concerns soil composition. *Cortinarius trivialis* is a common species which appears in different woods types, sclerophyllus, deciduous and conifers, and *Laccaria laccata* is also too common and abundant appearing in our country in all woods types (Pinho-Almeida and Baptista-Ferreira, 2005).

Thus, the analysis of these results can demonstrate that ECM species, even within the same genus, as *Russula amoenolens* and *Russula subfoetens*, responded differently to various nutrients. Different responses to nutrients were described in Twieg *et al*. (2009), where although specific taxa was positively related to soil available P, a negative relationship was found between this nutrient and ECM richness as a whole. Reverchon *et al*. (2012) also reported that species within the same fungal genus have different responses to soil variables.

Although both species belong to the same taxonomic series (Foetens) and some appear to be especially nitrophilic (Avis, 2012), *Russula amoenolens* does not seem to be related with N while *Russula subfoetens* appears to be favoured under poorer conditions of N, as well as O.M. In respect of N, the result obtained is not in agreement with Avis *et al*. (2003), who reported that *Russula* aff. *amoenolens* produces more sporocarps under high N supply.

Finally, this study demonstrates, generally, that higher levels O.M., and consequently the availability of N and P, appear to influence negatively the diversity and abundance of ECM species. Moreover, the species studied under the present work are likely to be adapted to different soil conditions.

Conclusions

Analysis of soil chemical parameters shows differences between study plots. Some of those, particularly relative to O.M., extractable P and N total concentrations, can be probably explained by the use different shrub control practices.

Concerning to macrofungal abundance, under the present study it is suggested that its quantification through the counting of the number of trees where certain species occur could be a useful alternative to the formal method used.

In all inventoried area were registered 706 individuals belonging to 35 genera and 132 taxa, from which 105 are ECM taxa. The most representative families were: Tricholomataceae, Russulaceae and Cortinariaceae, being *Laccaria laccata*, *Russula amoenolens* and *Cortinarius trivialis* the species more frequently registered.

In respect of evaluating macrofungal and ECM diversity, species richness seems to be a better diversity descriptor to evaluate the real situation observed among plots. The analysis of macrofungal and ECM abundance and diversity shows some differences among the study plots. BS and M were the plots with higher macrofungal and ECM abundance as well as diversity.

The results also demonstrate that the ectomycorrhizal fungi diversity was negatively correlated with extractable phosphorus concentration, which is also the nutrient that better explains the variability of the abundance. It is suggested that environments with increasing concentrations of this nutrient in the range “low level” (<25 mg kg^{-1}) may have a negative effect on the diversity and abundance of ECM fungi.

Furthermore, we can conclude that there are different behaviours among species in response to soil available nutrients.

It is important to continue characterizing the ECM fungal community associated with cork-oak in order to understand how it can be useful as indicator of ecological impacts in this ecosystem.

This study reveals some explanatory data for a better awareness about the influence of soil characteristics on the ectomycorrhizal fungal diversity and abundance. Additionally, the results attained suggest that different management practices may affect, both directly and indirectly, the diversity and abundance of ECM communities by damaging the roots and the mycorrhization process.

Therefore, among the management practices of cork-oak stands presently used, the adoption of measures that can improve ectomycorrhizal abundance and diversity should be encouraged. These aspects can guide future management options, enable the sustainability of the *montado* ecosystem, being of great importance under the present situation of decline of this ecosystem and the scenario of global climate change.

References

AFN (2010). 5º Inventário Florestal Nacional. Portugal Continental. 2005-2006. Lisboa.

Águeda, B., Parladé, J., Maria de Miguel, A., Martínez-Peña, F. (2006). Characterization and identification of field ectomycorrhizae of *Boletus edulis* and *Cistus ladanifer*. Mycologia 98: 23-30. Doi: 10.3852/mycologia.98.1.23

Ana-Magán, J. F., Fernández, A., R. (2000). Os Cogumelos nos Ecossistemas Florestais Galegos. Edicións Xerais de Galicia, S.A. A Coruña. 205 pp.

Aronson, J., Pereira, J. S., Pausas, J. G. (Eds). (2009). Cork Oak Woodlands on the Edge. Washington DC: Island Press. 315 pp.

Avis, P. G. (2012). Ectomycorrhizal iconoclasts: the ITS rDNA diversity and nitrophilic tendencies of fetid Russula. Mycologia 104:998-1007. doi: 10.3852/11-399

Avis, P. G., McLaughlin, D. J., Dentinger, B. C., Reich, P. B. (2003). Long-term increase in nitrogen supply alters aboveand below-ground ectomycorrhizal communities and increases the dominance of *Russula* spp. in a temperate oak savannah. New Phytologist 160: 239–253. doi: 10.1046/j.1469-8137.2003.00865.x

Azul, A. M. (2002). Diversidade de fungos ectomicorrízicos em ecossistemas de Montado. Tese de Doutoramento, Portugal. Universidade de Coimbra.

Azul, A. M. (2011). Diálogos e modos de actuação colectiva com vista à sustentabilidade do sobreiro em Portugal. Centro de Ecologia Funcional. Universidade de Coimbra. E-cadernos CES 10: 70-90.

Azul, A. M., Mendes, S. M., Sousa, J. P., Freitas, H. (2011). Fungal fruitbodies and soil macrofauna as indicators of land use practices on soil biodiversity in Montado. Agroforestry Systems 82: 121-138.

Azul, A. M, Ramos, V., Pato, A., Azenha, M., Freitas, H. (2008). Mycorrhizal types in the Mediterranean Basin: safety and teaching and training. Journal of Biological Education 42: 130-137.

Azul, A. M., Sousa, J. P., Agerer, R., Martín, M. P., Freitas, H. (2010). Land use practices and ectomycorrhizal fungal communities from oak woodlands dominated by *Q. suber* L. considering drought scenarios. Mycorrhiza 20: 73-88.

Baar, J., ter Braak, C. J. F. (1996). Ectomycorrhizal sporome occurance as affected by manipulation of litter and humus layers in Scots pine stands of different age. Applied Soil Ecology 4: 61-73.

Baptista, P., Martins, A., Tavares, R. M., Lino-Neto, T. (2010). Diversity and fruiting pattern of macrofungi associated with chestnut (*Castanea sativa*) in the Trás-os-Montes region (Northeast Portugal). Fungal Ecology 3: 9-19.

Barrico, L., Rodríguez-Echeverría, S., Freitas, H. (2010). Diversity of soil basidiomycete communities associated with *Quercus suber* L. Portuguese *montado*. European Journal of Soil Biology 46: 280-287.

Basso, M. T. (1999). Fungi Europaei – Lactarius Pers, Edizioni Candusso (Mykoflora, I – Alassio). 845 pp.

Bon, M. (1988). Champignons d'Europe Occidentale. Les Editions Arthaud. 368 pp.

Bugalho, M.N., Caldeira, M. C., Pereira, J. S., Aronson, J., Pausas, J. G. (2011). Mediterranean cork-oak savannas require human use to sustain biodiversity and ecosystem services. Frontiers in Ecology and the Environment. doi: 10.1890/100084

Buscardo, E., Rodríguez-Echeverría, S., Martín, M. P., De Angelis, P., Pereira, J. S., Freitas, H. (2010). Impact of wildfire return interval on the ectomycorrhizal resistantpropagules communities of a Mediterranean open forest. Fungal Biology 114: 628-636.

Cañellas, I., Roig, S., Poblaciones, MJ., Gea-Isquierdo, G., Olea, L. (2007). An approach to acorn production in Iberian dehesas. Agroforestry Systems 70: 3-9.

Cardoso, I.M., Kuyper, T.W. (2006). Mycorrhizas and tropical soil fertility. Agriculture, Ecosystems and Environment 116: 72-84.

Cardoso, J. V., de C. (1965). Os Solos de Portugal, sua Classificação, Caracterização e Génese: 1 – A Sul do Rio Tejo. Secretaria de Estado da Agricultura. Direcção-Geral dos Serviços Agrícolas. Lisboa.

Carrete, M., Donázar J. A. (2005). Application of central-place foraging theory shows the importance of Mediterranean dehesas for the conservation of the cinereous vulture, *Aegypius monachus.* Biological Conservation 126: 582–590. In: Bugalho *et al*. (2011).

Castro, H., Lehsten, V., Lavorel, S., Freitas, H. (2010). Functional response traits in relation to land use change in the Montado. Agriculture, Ecosystems and Environment 137 : 183-191.

Costa, A., Pereira, H., Madeira, M. (2009). Landcape dynamics in endangered cork-oak woodlands in southwestern Portugal (1958-2005). Agroforestry Systems 77: 83-96. Doi 10.1007/s10457-009-9212-3.

Costa, J. V. B. (2004). Caracterização e Constituição do Solo. 7th edition. Fundação Calouste Gulbenkian. 527 pp.

Courtecuisse, R., Bernard, D. (2000). Guide des Champignons de France et d'Europe. Delachaux et Niesttlé (Ed.). Paris. 476 pp.

Díaz, M., Campos, P., Pulido, F. (1997). The Spanish dehesas: a diversity of land uses and wildlife. In: Pain, D., Pienkowski, M. (1997). (eds). Farming and Birds in Europe: the common agricultural policy and its implications for bird conservation. Academic Press, London, UK. *cited in*: Bugalho *et al*. (2011).

Díaz-Villa, M. D., Marañon, T., Arroyo, J. (2003). Soil seed bank and floristic diversity in a forest-grassland mosaic in southern Spain. J Veg Sci 14: 701-709. *cited in*: Bugalho *et al*. (2011).

Edmonds, R.L., Agee, J.K., Gara, R.I. (2000). Forest Health and Protection. 2nd ed. Waveland Press, Inc., USA, 667 pp.

FAO. 2006. Guidelines for Soil Description. 4th edition FAO, Rome.

FAO–ISRIC. 1990. Guidelines for Profile Description. 3rd Edition. Rome *cited in*: FAO. 2006. Guidelines for soil description. 4th edition FAO, Rome.

Fisher, R. F., Binkley, D. (2000). Ecology and Management of Forest Soils. 3rd ed. John Wiley & Sons, Inc. USA 489 pp.

Gardes, M., Bruns, T. D. (1996). Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: above- and below-ground views. Canadian Journal of Botany 74: 1572-1583. *cited in*: Reverchon *et al*. (2012).

Garrett, S. D. (1981). Soil Fungi and Soil Fertility. 2nd ed. Pergamon Press. England. 150 pp.

Gerhardt, E., Vila, J., Llimona, X. 2000. Hongos de España y de Europa. Omega Eds., Barcelona, 957 pp.

Hacskaylo, E. (1973). Carbohydrate Physiology of Ectomycorrhizae. In: Marks, G. C., Foster, R. C. (1973). Structure, Morphogenesis, and Ultrastructure of Ectomycorrhizae. In: Marks, G. C. and Kozlowski, T. T. (1973).

Harley J. L., Smith, S. E. (1983). Mycorrhizal Symbiosis. Academic Press. c*ited in*: Martins, A. R. L. (2004).

INMG (Instituto Nacional de Meteorologia e Geofísica) (1991). Normais Climatológicas da região de "Alentejo e Algarve" correspondentes a 1951-1980. O Clima de Portugal. Lisboa.

Instituto Mediterrânico. Universidade Nova de Lisboa. (1998). Desertificação. Nos 12/13 Semestral. 326 pp.

Inverno C. M. C., Manuppella G., Zbyszewski, G. (1993). Carta Geológica de Portugal na escala 1:50000 -42 C. Notícia explicativa da Folha 42 C. Santiago do Cacém. Serviços Geológicos de Portugal. Lisboa. *cited in*: Costa *et al*. (2009).

IUSS Working group WRB (2006). World Reference Base for Soil Resources 2006. 2nd edition. World Soil Resources Reports No. 103. FAO, Rome.

Joffre, R., Rambal, S. and Ratte, J. P. (1999). The dehesa system of southern Spain and Portugal as a natural ecosystem mimic. Agroforestry Systems 45: 57-79.

Joffre, R., Vacher, J., de los Llamos, C., Long, G. (1988). The dehesa: an agrosilvopastoral system of the Mediterranean region with special reference to the Sierra Morena area of Spain. Agroforestry Systems 6: 71-96. *cited in*: Cañellas *et al*. (2007).

Killhan, K. (1994). Soil Ecology. Cambridge University Press. 242 pp.

Kranabetter, J. M., Durall, D. M., MacKenzie, W. H.. (2009). Diversity and species distribution of ectomycorrhizal fungi along productively gradients of a southern boreal forest. Mycorrhiza 19: 99-111.

Landeweert, R., Leeflang, P., Smit, E., Kuyper, T. (2005). Diversity of an ectomycorrhizal fungal community studied by a root tip and total soil DNA approach. Mycorrhiza 15:1-6. *cited in*: Barrico *et al*. (2010).

Lourenço, N., Pinto-Correia, T., Jorge, M. R. (1998). Farming strategies and land use changes in Southern Portugal: land abandonment or extensification of the traditional systems. In: Instituto Mediterrânico. Universidade Nova de Lisboa. (1998).

Lozano, F. P. (2001) Setas para Todos. Guia Práctica de Identificación. Editorial PIRINEO. 653 pp.

LQARS, Laboratório Químico Agrícola Rebelo da Silva (2006). Manual de fertilizações das culturas. INIAP. Lisboa.282 pp.

Magurran, A. E. (1988). Ecological Diversity and its Measurement. Princeton University Press. New Jersey. 179 pp. *cited in*: Moreno, C. E. (2001).

Marañon, T. (1996). Plant species richness and canopy effect in the savanna-like “dehesa” of SW Spain. Ecología Mediterránea 12: 131-141. *cited in*: Plieninger, T. and Wildbrand, C. (2001)

Marks, G. C., Foster, R. C. (1973). Structure, Morphogenesis, and Ultrastructure of Ectomycorrhizae. In: Marks, G. C., Kozlowski, T. T. (1973). Ectomycorrhizae. Academic Press, Inc. New York. 444 pp.

Marks, G. C., Kozlowski, T. T. (1973). Ectomycorrhizae. Academic Press, Inc. New York. 444 pp.

Marôco, J. (2011). Análise Estatística com o SPSS Statistics. 5ª Ed. ReportNumber, Lda. Pero Pinheiro. 990 pp.

Martin-Vicent, A., Fernández-Alés, R. (2006). Long term persistente of dehesas. Evidences from history. Agroforestry Systems 67: 19-28. *cited in*: Cañellas *et al*. (2007).

Martins, A. R. L. (2004). Micorrização controlada de *Castanea sativa* Mill.: Aspectos fisiológicos da micorrização *in vitro* e *ex vitro*. Dissertação de Doutoramento. Faculdade de Ciências da Universidade de Lisboa. Lisboa. 566 pp.

Marx, D. H. (1977). The role of mycorrhizae in forest production. Pp. 151-161. In: TAPPI conference papers. Atlanta, GA. c*ited in* : Fisher, R. F. and Binkley, D. (2000).

Montoya, J. M. (1980). Los Alcornocales. Instituto Nacional de Investigaciones Agrarias. Ministerio de Agricultura. Madrid. 155 pp.

Moreau P.-A., Daillant O., Corriol G., Gueidan C., Courtecuisse R. (2002) RENECOFOR – Inventaire des champignons supérieurs et des lichen sur 12 placettes du réseau et dans un site atelier de 1'INRA/GIP ECOFOR – Résultats d'un project pilote (1996-1998). Editeur: Office National des Forêts, Département Recherche et Développement, ISBN 2 – 84207 - 244 – 8, 142p.

Moreira, A. C., Martins, J. M. S. (2005). Influence of site factors on the impact of *Phytophthora cinnamoni*. In: cork-oak stands in Portugal. Forest Pathology 35: 145-162. *cited in*: Serrasolses *et al*. (2009).

Moreno, C. E. (2001). Métodos para medir la diversidade. M&T – Manuales y Tesis SEA, vol. 1. Zaragoza, 84 pp.

Mousain, D., Boukcim, H., Richard, F. (2009). Mycorrhizal Symbiosis and Its Role in Seedling Response to Drought In: Aronson *et al*. (2009).

Munõz, J. A. (2005). Fungi Europaei - Boletus s.l. (excl. Xerocomus), Edizioni Candusso. 952 pp.

Munsell. (1954). Munsel soil color carth. Munsell color company. Inc. Baltimore 2. Maryland. USA.

Natividade, J. V. (1990). Subericultura. 2ª ed. Ministério da Agricultura, Pescas e Alimentação. Direcção-Geral das Florestas. Lisboa.

Ortega., A., Lorite, J. (2007). Macrofungi diversity in cork-oak and holm-oak forests in Andalusia (southern Spain); an efficient parameter for establishing priorities for its evaluation and conservation. Central European Journal of Biology 2: 276-296.

Otieno, D. O., Mirzae, H., Hussain, M. Z., Li, Y. L., Schmidt, M. W. T., Wartinger, M., Jung, E., Ribeiro, N., Pereira, J. S., Tenhunen, J. (2011). Herbaceous layer development during spring does not deplete soil nitrogen in Portuguese montado. Journal of Arid Environments 75: 231-238.

Pausas J. G., Pereira, J. S., Aronson, J. (2009). The Tree. In: Aronson *et al*. (2009).

Pelczar, M. J., Chan, E. C. S., Krieg, N. R. (1986). Microbiology. 5th ed. McGraw-Hill, Inc. USA. 918 pp.

Peter, M., Ayer, F., Egli, S., Honegger, R. (2001). Above- and below-ground community structure of ectomycorrhizal fungi in three Norway spruce (*Picea abies*) stands in Switzerland. Canadian Journal of Botany 79: 1134-1151. *cited in*: Reverchon *et al*. (2012).

Pinho-Almeida, F., Baptista-Ferreira, J. L. (2005). Cogumelos da Barrosinha (Alcácer do Sal) – Inventarição de macrofungos em áreas seleccionadas. Centro de Micologia da Universidade de Lisboa. 119 pp.

Pinto-Correia,T. (1993). Threatened Landscape in Alentejo, Portugal. The "montado" and other "agro-silvo pastoral systems". Landscape Urban Planning 24: 43-48. *cited in*: Azul, A. M. (2011).

Pinto-Correia,T., Mascarenhas, J. (1999). Contribution to the extensification/intensification debate: new trends in the Portuguese *montado*. Landscape Urban Planning 46: 125-131.

Plieninger, T., Wildbrand, C. (2001). Land use, biodiversity, conservation, and rural development in the dehesas of Cuatro Lugares, Spain. Agroforestry Systems 51: 23

Rebelo, R., Correia, A. I., Fonseca, F., Mathias, M. L., Santos-Reis, M. (2009). Herdade da Ribeira Abaixo e Serra de Grândola. Ecossistemas.indd. Capítulo 18: 637-659.

Reverchon, F., Ortega-Larrocea, M. P., Pérez-Moreno, J. (2012). Soil factors influencing ectomycorrhizal sporome distribution in neotropical forests dominated by *Pinus montezumae*, Mexico. Mycoscience 53: 203-210. DOI 10.1007/s10267-011-0136-1.

Richard, F., Millot, S., Gardes, M., Selosse, M. A. (2005). Diversity and specificity of ectomycorrhizal fungi retrieved from an old-growth Mediterranean forest dominated by *Quercus ilex*. New Phytologist 166: 1011-1023. *cited in*: Reverchon *et al*. (2012).

Richard, F., Moreau, P. A., Selosse, M. A., Gardes, M. (2004). Diversity and fruiting patterns of ectomycorrhizal and saprobic fungi in an old-growth Mediterranean forest dominated by *Quercus ilex* L. Canadian Journal of Botany 82: 1711-1729. *cited in* Mousain *et al*. (2009).

Ruiz de la Torre, J. (2002). Árboles y Arbustos de la España Peninsular. Fundación Conde del Vallede Salazar. E.T.S. de Ingenieros de Montes, Mundi-Prensa Libros S.A., Madrid. Spain. c*ited in*: Serrasolses *et al*. (2009).

San Miguel, A. (1994). La dehesa española: origem, tipología, características y gestíon. Fundacíon Conde del Valle de Salazar, Madrid. *cited in*: Cañellas *et al*. (2007).

Sánchez, L. A. P. (2008). Fungi Europaei – Agaricus L. Allopsalliota Nauta & Bas Tribu Agariceae S. Imai Part I, Edizioni Candusso. 824 pp.

Sánchez-Palomares, O., Jovellar, L. C., Sarmiento, A., Rubio, A., Gandullo, A. (2007). Las Estaciones Ecológicas de los Alcornocales Españoles. Monografías INIA: Serie Forestal, no. 14, Ministerio de Educación y Ciencia, Madrid, Spain. *cited in*: Serrasolses *et al*. (2009).

Santos, N. F. (1948). Micorrizas no sobreiro. Rev. Agronómica 36 (2) *cited in*: Natividade J. V. (1990).

Santos-Silva, C., Gonçalves, A., Louro, R. (2011). Canopy cover influence on macrofungal richness and sporocarp production in montado ecosystems. Agroforestry Systems 82: 149-159.

Sarnari M. (2005). Monografia Illustrata del Genere Russula in Europa Vol. 2, A.M.B. Fondazione Centro Studi Micologici, 1568 pp.

Sarnari M. (2007). Monografia Illustrata del Genere Russula in Europa Vol. 1**,** A.M.B. Fondazione Centro Studi Micologici, 799 pp.

Serrasolses, I., Pérez-Devesa, M., Vilagrosa, A., Pausas, J. G., Sauras, T., Cortina, J., Vallejo, V. R. (2009). Soil Properties Constraining Cork Oak Distribution. In: Aronson *et al*. (2009).

Schmit, J. P., Murphy, J. F., Mueller, G. M. (1999). Macrofungal diversity of a temperate oak forest: a test of species richness estimators. Canadian Journal of Botany 77: 1014-1027.

Smith, M. E., Douhan, G.W., Rizzo, D.M. (2007). Ectomycorrhizal community structure in a xeric Quercus woodland based on rDNA sequence analysis of sporocarps and pooled roots. New Phytologist 174: 847-863. *cited in*: Mousain *et al*. (2009).

Smith, S. E., Read D. J. (2008). Mycorrhizal Simbiosis. 3rd edition. Academic press. 787 pp.

Soveral-Dias, J. C., Santos, A. D., Nogueira, M. G. B., Tavares, M. M. V. S. (1980). Guia Prático de Fertilização. Serviço de Análise de terras e de Análise Foliar. DGER, Laboratório Químico Rebelo da Silva, Lisboa, 72 pp. *cited in*: LQARS, Laboratório Químico Agrícola Rebelo da Silva (2006).

Torres Juan, J. (1975). Patologia Forestall. E. T. S. I. Montes, Madrid. *cited in* Montoya, J. M. (1980).

Torres, P., Roldan, A., Lansac, A. R., Martin, A. (1995). Ectomycorrhizal formation between Cistus ladanifer and *Laccaria-laccata*. Nova Hedwigia 60: 311-315 *cited in*: Águeda *et al*. (2006).

Twieg, B. D., Durall, D. M, Simard, S. W., Jones, M. D. (2009). Influence of soil nutrients on ectomycorrhizal communities in a chronosequence of mixed temperate forests. Mycorrhiza 19: 305-316. *cited in* Reverchon *et al*. (2012).

Annex 1

6. INVENTÁRIO MICOLÓGICO

2. ESTADO DO TEMPO	NEBULOSIDADE	PRECIPITAÇÃO

AMOSTRA Nº	DADOS ÁRVORE				DADOS ESPÉCIE			OBS
	Nº ÁRVORE	Distância à árvore (m)	ORIENTAÇÃO	Nº FOTO	ESPÉCIE	Nº CARPÓFOROS	INDIVIDUOS / GRUPOS	FLORA / LOCALIZAÇÃO / NOTAS

Annex 2

Outputs of tests of normality and non parametric Kruskall-Wallis and Mann-Whitney tests to examine differences among study plots (Plot 1: BS; Plot 2: M; Plot 3: VP; Plot 4: TN) relatively to soil parameters (Extractable phosphorus: Ext P; Extractable potassium: Ext K; organic matter: OM; pH; Total nitrogen: Total_N)

Tests of Normality

	PLOT	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Ext P	1	,283	4	.	,877	4	,328
	2	,386	4	.	,774	4	,063
	3	,284	4	.	,874	4	,315
	4	,325	4	.	,876	4	,321
Ext K	1	,252	4	.	,930	4	,595
	2	,260	4	.	,832	4	,174
	3	,198	4	.	,973	4	,862
	4	,237	4	.	,969	4	,833
OM	1	,274	4	.	,843	4	,203
	2	,193	4	.	,974	4	,863
	3	,240	4	.	,876	4	,324
	4	,258	4	.	,957	4	,762
pH	1	,218	4	.	,920	4	,538
	2	,298	4	.	,849	4	,224
	3	,441	4	.	,630	4	,001
	4	,333	4	.	,763	4	,051
Total_N	1	,185	4	.	,967	4	,823
	2	,204	4	.	,967	4	,824
	3	,248	4	.	,870	4	,298
	4	,361	4	.	,791	4	,087

a. Lilliefors Significance Correction

Test of Homogeneity of Variance

		Levene Statistic	df1	df2	Sig.
Ext P	Based on Mean	,844	3	12	,496
	Based on Median	,698	3	12	,571
	Based on Median and with adjusted df	,698	3	10,143	,574
	Based on trimmed mean	,839	3	12	,498
Ext K	Based on Mean	,290	3	12	,832
	Based on Median	,262	3	12	,852
	Based on Median and with adjusted df	,262	3	8,101	,851
	Based on trimmed mean	,290	3	12	,832
OM	Based on Mean	4,993	3	12	,018
	Based on Median	3,656	3	12	,044
	Based on Median and with adjusted df	3,656	3	3,138	,151
	Based on trimmed mean	4,973	3	12	,018
pH	Based on Mean	1,044	3	12	,408
	Based on Median	,923	3	12	,459
	Based on Median and with adjusted df	,923	3	10,088	,464
	Based on trimmed mean	1,047	3	12	,407
Total_N	Based on Mean	2,874	3	12	,080
	Based on Median	1,107	3	12	,385
	Based on Median and with adjusted df	1,107	3	5,766	,419
	Based on trimmed mean	2,551	3	12	,105

Kruskal-Wallis Test

Ranks

	PLOT	N	Mean Rank
Ext P	1	4	6,50
	2	4	2,50
	3	4	14,50
	4	4	10,50
	Total	16	
Ext K	1	4	14,50
	2	4	2,50
	3	4	6,75
	4	4	10,25
	Total	16	
OM	1	4	9,50
	2	4	2,50
	3	4	14,25
	4	4	7,75
	Total	16	
pH	1	4	14,38
	2	4	3,75
	3	4	9,13
	4	4	6,75
	Total	16	
Total_N	1	4	8,25
	2	4	2,50
	3	4	14,50
	4	4	8,75
	Total	16	

Test Statistics[a,b]

	Ext P	Ext K	OM	pH	Total_N
Chi-Square	14,118	13,787	12,463	10,858	12,728
df	3	3	3	3	3
Asymp. Sig.	,003	,003	,006	,013	,005

a. Kruskal Wallis Test

b. Grouping Variable: PLOT

Mann-Whitney Test

Ranks

	PLOT	N	Mean Rank	Sum of Ranks
Ext P	1	4	6,50	26,00
	2	4	2,50	10,00
	Total	8		
Ext K	1	4	6,50	26,00
	2	4	2,50	10,00
	Total	8		
pH	1	4	6,50	26,00
	2	4	2,50	10,00
	Total	8		
Total_N	1	4	6,50	26,00
	2	4	2,50	10,00
	Total	8		
OM	1	4	6,50	26,00
	2	4	2,50	10,00
	Total	8		

Test Statistics[a]

	Ext P	Ext K	pH	Total_N	OM
Mann-Whitney U	,000	,000	,000	,000	,000
Wilcoxon W	10,000	10,000	10,000	10,000	10,000
Z	-2,309	-2,309	-2,323	-2,309	-2,309
Asymp. Sig. (2-tailed)	,021	,021	,020	,021	,021
Exact Sig. [2*(1-tailed Sig.)]	,029[b]	,029[b]	,029[b]	,029[b]	,029[b]

a. Grouping Variable: PLOT

b. Not corrected for ties.

Mann-Whitney Test

Ranks

	PLOT	N	Mean Rank	Sum of Ranks
Ext P	1	4	2,50	10,00
	3	4	6,50	26,00
	Total	8		
Ext K	1	4	6,50	26,00
	3	4	2,50	10,00
	Total	8		
pH	1	4	6,50	26,00
	3	4	2,50	10,00
	Total	8		
Total_N	1	4	2,50	10,00
	3	4	6,50	26,00
	Total	8		
OM	1	4	2,75	11,00
	3	4	6,25	25,00
	Total	8		

Test Statistics[a]

	Ext P	Ext K	pH	Total_N	OM
Mann-Whitney U	,000	,000	,000	,000	1,000
Wilcoxon W	10,000	10,000	10,000	10,000	11,000
Z	-2,309	-2,309	-2,366	-2,309	-2,021
Asymp. Sig. (2-tailed)	,021	,021	,018	,021	,043
Exact Sig. [2*(1-tailed Sig.)]	,029[b]	,029[b]	,029[b]	,029[b]	,057[b]

a. Grouping Variable: PLOT

b. Not corrected for ties.

Mann-Whitney Test

Ranks

	PLOT	N	Mean Rank	Sum of Ranks
Ext P	1	4	2,50	10,00
	4	4	6,50	26,00
	Total	8		
Ext K	1	4	6,50	26,00
	4	4	2,50	10,00
	Total	8		
pH	1	4	6,38	25,50
	4	4	2,63	10,50
	Total	8		
Total_N	1	4	4,25	17,00
	4	4	4,75	19,00
	Total	8		
OM	1	4	5,25	21,00
	4	4	3,75	15,00
	Total	8		

Test Statistics[a]

	Ext P	Ext K	pH	Total_N	OM
Mann-Whitney U	,000	,000	,500	7,000	5,000
Wilcoxon W	10,000	10,000	10,500	17,000	15,000
Z	-2,309	-2,309	-2,191	-,289	-,866
Asymp. Sig. (2-tailed)	,021	,021	,028	,773	,386
Exact Sig. [2*(1-tailed Sig.)]	,029[b]	,029[b]	,029[b]	,886[b]	,486[b]

a. Grouping Variable: PLOT

b. Not corrected for ties.

Mann-Whitney Test

Ranks

	PLOT	N	Mean Rank	Sum of Ranks
Ext P	2	4	2,50	10,00
	3	4	6,50	26,00
	Total	8		
Ext K	2	4	2,50	10,00
	3	4	6,50	26,00
	Total	8		
pH	2	4	2,75	11,00
	3	4	6,25	25,00
	Total	8		
Total_N	2	4	2,50	10,00
	3	4	6,50	26,00
	Total	8		
OM	2	4	2,50	10,00
	3	4	6,50	26,00
	Total	8		

Test Statistics[a]

	Ext P	Ext K	pH	Total_N	OM
Mann-Whitney U	,000	,000	1,000	,000	,000
Wilcoxon W	10,000	10,000	11,000	10,000	10,000
Z	-2,309	-2,309	-2,084	-2,309	-2,309
Asymp. Sig. (2-tailed)	,021	,021	,037	,021	,021
Exact Sig. [2*(1-tailed Sig.)]	,029[b]	,029[b]	,057[b]	,029[b]	,029[b]

a. Grouping Variable: PLOT

b. Not corrected for ties.

Mann-Whitney Test

Ranks

	PLOT	N	Mean Rank	Sum of Ranks
Ext P	2	4	2,50	10,00
	4	4	6,50	26,00
	Total	8		
Ext K	2	4	2,50	10,00
	4	4	6,50	26,00
	Total	8		
pH	2	4	3,50	14,00
	4	4	5,50	22,00
	Total	8		
Total_N	2	4	2,50	10,00
	4	4	6,50	26,00
	Total	8		
OM	2	4	2,50	10,00
	4	4	6,50	26,00
	Total	8		

Test Statistics[a]

	Ext P	Ext K	pH	Total_N	OM
Mann-Whitney U	,000	,000	4,000	,000	,000
Wilcoxon W	10,000	10,000	14,000	10,000	10,000
Z	-2,309	-2,309	-1,191	-2,309	-2,309
Asymp. Sig. (2-tailed)	,021	,021	,234	,021	,021
Exact Sig. [2*(1-tailed Sig.)]	,029[b]	,029[b]	,343[b]	,029[b]	,029[b]

a. Grouping Variable: PLOT

b. Not corrected for ties.

Mann-Whitney Test

Ranks

	PLOT	N	Mean Rank	Sum of Ranks
Ext P	3	4	6,50	26,00
	4	4	2,50	10,00
	Total	8		
Ext K	3	4	2,75	11,00
	4	4	6,25	25,00
	Total	8		
pH	3	4	5,38	21,50
	4	4	3,63	14,50
	Total	8		
Total_N	3	4	6,50	26,00
	4	4	2,50	10,00
	Total	8		
OM	3	4	6,50	26,00
	4	4	2,50	10,00
	Total	8		

Test Statistics[a]

	Ext P	Ext K	pH	Total_N	OM
Mann-Whitney U	,000	1,000	4,500	,000	,000
Wilcoxon W	10,000	11,000	14,500	10,000	10,000
Z	-2,309	-2,021	-1,049	-2,309	-2,309
Asymp. Sig. (2-tailed)	,021	,043	,294	,021	,021
Exact Sig. [2*(1-tailed Sig.)]	,029[b]	,057[b]	,343[b]	,029[b]	,029[b]

a. Grouping Variable: PLOT

b. Not corrected for ties.

Annex 3

Output of normality test and linear regression to study the existence of relationship between number of fruiting bodies (n1) and number of individuals (n2).

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Log n1	,098	80	,054	,954	80	,006

a. Lilliefors Significance Correction

Output of normality tests to study relation between diversity indices (S, H' and D) and soil parameters.

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
S	,123	12	,200*	,944	12	,545
D	,162	12	,200*	,932	12	,399
H´	,153	12	,200*	,941	12	,506

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Output of normality tests to study of association of the ectomycorrhizal abundance and soil parameters

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Myc trees (%)	,135	12	,200*	,938	12	,471
trees with Ct (%) t	,209	12	,155	,880	12	,087
trees with Ll (%)	,240	12	,056	,863	12	,053
trees with Ra (%) t	,220	12	,111	,874	12	,073
trees with Rs (%)	,178	12	,200*	,918	12	,271

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Annex 4

List of species, number of fruiting bodies (n1) and number of individuals (n2) registered in the sampling plots.

BS – Barradas da Serra; M – Mostardeira; TN – Tanganhal Novo; VP – Várzea dos Pereiros

Macrofungal taxa	Family	Mycorrhizal	Sampling plots							
			BS		M		TN		VP	
			n1	n2	n1	n2	n1	n2	n1	n2
Agaricus sp. 1	Agaricaceae	No/sap	2	1						
Agaricus sp. 3	Agaricaceae	No/sap			1	1				
Agaricus sp. 4	Agaricaceae	No/sap					1	1		
Amanita caesarea (Scop.: Fr.) Pers.	Amanitaceae	Yes	15	9	1	1				
Amanita citrina (Schaeff.: Fr.) Gray	Amanitaceae	Yes	6	2						
Amanita fulva (Schaeff.: Fr.) Fr.	Amanitaceae	Yes			2	2				
Amanita mairei Foley	Amanitaceae	Yes	1	1	3	2			1	1
Amanita pantherina (DC.: Fr.) Krombh.	Amanitaceae	Yes	11	4	1	1			1	1
Amanita phalloides (Vaill.: Fr.) Link	Amanitaceae	Yes	3	3						
Amanita rubescens Gillet	Amanitaceae	Yes	3	2	4	2				
Amanita submembranacea (M. Bon) Gr öger	Amanitaceae	Yes			4	1				
Amanita vaginata (Schaeff.) Vesely	Amanitaceae	Yes	8	7	21	13				
Armillaria gallica Merxm. & Romagn.	Physalacriaceae	No/pat	12	3						
Astraeus hygrometricus (Pers.) Morgan	Diplocystidiaceae	Yes			1	1			1	1
Auricularia auricula-judae (Bull.) Quél.	Auriculariaceae	No/sap							5	1
Boletus aereus Bull. : Fr.	Boletaceae	Yes	4	2	36	21			3	2
Boletus aestivalis (Paulet) Fr.	Boletaceae	Yes	2	2	5	2				
Boletus fragrans Vittad.	Boletaceae	Yes	1	1						
Boletus queletii Schulzer	Boletaceae	Yes			1	1				
Clitocybe costata Kühner & Romagn.	Tricholomataceae	No/sap	41	4	41	2				
Clitocybe gibba (Pers.: Fr.) P. Kummer	Tricholomataceae	No/sap	4	2						
Clitocybe odora (Bull.: Fr.) P. Kummer	Tricholomataceae	No/sap	2	2	2	1				
Clitopilus prunulus (Scop.) P. Kumm.	Entolomataceae	Yes			2	1				
Collybia butyracea (Bull.: Fr.) P. Kummer	Marasmiaceae	Yes			3	1				
Collybia dryophila (Bull.:Fr.) P. Kummer	Marasmiaceae	No/sap			12	1	3	1		
Coprinus picaceus (Bull.: Fr.) Gray	Agaricaceae	No/sap					1	1	1	1
Cortinarius decipiens (Pers.: Fr.) Fr.	Cortinariaceae	Yes			28	5	10	1	2	1

Macrofungal taxa	Family	Mycorrhizal	Sampling plots							
			BS		M		TN		VP	
			n1	n2	n1	n2	n1	n2	n1	n2
Cortinarius elatior Fr.	Cortinariaceae	Yes	62	17	4	1				
Cortinarius orellanus (Fr.) Fr.	Cortinariaceae	Yes			54	16			14	3
Cortinarius trivialis J.E.Lange	Cortinariaceae	Yes	166	29	54	8			1	1
Cortinarius variecolor (Pers.: Fr.) Fr.	Cortinariaceae	Yes	3	2						
Cortinarius sp. 1	Cortinariaceae	Yes	1	1						
Cortinarius sp. 2	Cortinariaceae	Yes	1	1						
Cortinarius sp. 3	Cortinariaceae	Yes	4	1	9	2				
Entoloma lividum (Bull.) Quél.	Entolomataceae	Yes	1	1	3	3				
Entoloma sordidulum (Kühner & Romagn.) P.D.Orton	Entolomataceae	No/sap	5	2	23	6				
Gymnopilus penetrans (Fr.:Fr.) Murrill	Strophariaceae	No/sap			4	1	11	2	8	1
Gymnopilus spectabilis (Fr.: Fr.) Smith	Strophariaceae	No/sap							13	2
Gymnopilus suberis (R. Maire) Singer	Strophariaceae	No/sap			2	2	31	2		
Hebeloma crustuliniforme (Bull.: Fr.) Quél.	Strophariaceae	Yes			36	9			4	2
Hebeloma sp. 1	Strophariaceae	Yes	1	1						
Helvella lacunosa Afzel.	Helvellaceae	No/sap							3	1
Hygrophorus cossus (Sowerby: Fr.) Fr.	Hygrophoraceae	Yes	11	3						
Hygrophorus discoxanthus Rea	Hygrophoraceae	Yes	63	13						
Hygrophorus eburneus (Bull.: Fr.) Fr.	Hygrophoraceae	Yes	1	1						
Hygrophorus latitabundus Britzelm.	Hygrophoraceae	Yes	10	1						
Hypholoma fasciculare (Huds.:Fr.) P. Kumm.	Strophariaceae	No/sap	17	1						
Inocybe geophylla (Peck) Gillet	Inocybaceae	Yes							1	1
Inocybe lacera (Fr.: Fr.) P. Kumm.	Inocybaceae	Yes	5	1						
Inocybe piriodora (Pers.: Fr.) P. Kumm.	Inocybaceae	Yes	4	1						
Inocybe sp. 1	Inocybaceae	Yes			2	1				
Inocybe sp. 2	Inocybaceae	Yes			10	2				
Laccaria laccata (Scop.: Fr.) Cooke	Tricholomataceae	Yes	285	31	622	53			152	12
Lactarius acerrimus Britzelm.	Russulaceae	Yes	6	1						

Macrofungal taxa	Family	Mycorrhizal	Sampling plots							
			BS		M		TN		VP	
			n1	n2	n1	n2	n1	n2	n1	n2
Lactarius atlanticus Bon	Russulaceae	Yes							3	3
Lactarius chrysorrheus Fr.	Russulaceae	Yes	20	5	30	3			3	3
Lactarius decipiens Quél.	Russulaceae	Yes	1	1	5	3				
Lactarius deliciosus (L.:Fr.) Gray	Russulaceae	Yes	46	9						
Lactarius rugatus Kühner & Romagn.	Russulaceae	Yes	19	2	11	6			2	1
Lactarius semisanguifluus R. Heim & Leclair	Russulaceae	Yes	1	1						
Lactarius serifluus (DC.: Fr.) Fr.	Russulaceae	Yes			13	3				
Lactarius zonarius (Bull.) Fr.	Russulaceae	Yes	7	1						
Lactarius sp. 1	Russulaceae	Yes	1	1						
Lepiota sp. 1	Agaricaceae	No/sap					12	1		
Lycoperdon lambinonii Demoulin	Agaricaceae	No/sap			2	1				
Lycoperdon perlatum Pers.	Agaricaceae	No/sap			15	7				
Macrolepiota fuliginosa (Barla) Bon	Agaricaceae	No/sap					6	1	7	1
Macrolepiota procera (Scop.: Fr.) Singer	Agaricaceae	No/sap			4	1	9	1	21	8
Omphalotus olearius (DC.: Fr.) Fayod	Marasmiaceae	No/sap	1	1	4	3				
Phallus impudicus L.: Pers.	Phallaceae	No/sap							1	1
Pisolithus arhizus (Scop.) Rauschert	Sclerodermataceae	Yes			1	1				
Pluteus sp. 1	Pluteacea	No/sap					1	1		
Psathyrella sp.	Psathyrellaceae	No/sap					18	1	1	1
Russula albonigra Krombh.	Russulaceae	Yes	6	1	2	1				
Russula amoenicolor Romagn.	Russulaceae	Yes	6	2						
Russula amoenolens Romagn.	Russulaceae	Yes			124	25			40	10
Russula aurea Pers.	Russulaceae	Yes	4	3						
Russula cessans A. Pearson	Russulaceae	Yes			23	8				
Russula chloroides Krombh.	Russulaceae	Yes	1	1	38	9				
Russula cutrefacta Cooke	Russulaceae	Yes	2	2	1	1				
Russula cyanoxantha (Schaeff.) Fr.	Russulaceae	Yes	9	6	10	5				

Macrofungal taxa	Family	Mycorrhizal	Sampling plots							
			BS		M		TN		VP	
			n1	n2	n1	n2	n1	n2	n1	n2
Russula delica Fr.	Russulaceae	Yes	16	4	27	5				
Russula faginea Romagn. Ex Adamcík	Russulaceae	Yes			9	4				
Russula foetens Pers.: Fr.	Russulaceae	Yes			2	2				
Russula fragilis (Pers.: Fr.) Fr.	Russulaceae	Yes			9	3			2	2
Russula fuscorubroides Bon	Russulaceae	Yes	1	1						
Russula grisea (Pers.) Fr.	Russulaceae	Yes			1	1				
Russula illota Romagn.	Russulaceae	Yes	3	2	4	2				
Russula ionochlora Romagn.	Russulaceae	Yes	2	1	3	1				
Russula langei Bon	Russulaceae	Yes	3	2	9	2				
Russula lepida (Fr.: Fr.) Fr.	Russulaceae	Yes	3	1						
Russula nigricans (Bull.) Fr.	Russulaceae	Yes	5	3						
Russula pectinatoides Peck	Russulaceae	Yes			3	1				
Russula praetervisa Sarnari	Russulaceae	Yes			2	1				
Russula pseudopuellaris (Bon) Bon	Russulaceae	Yes	1	1						
Russula sororia (Fr.) Romell	Russulaceae	Yes	2	2						
Russula subfoetens W.G.Smith	Russulaceae	Yes	17	8	39	14			5	2
Russula sanguinea (Bull.) Fr. Syn. *Russula sulphurea Velen.*	Russulaceae	Yes			2	1				
Russula vesca Fr.	Russulaceae	Yes	49	13	4	2				
Russula violeipes Quél.	Russulaceae	Yes	5	4	38	6				
Russula sp. 1	Russulaceae	Yes			11	2				
Russula sp. 2	Russulaceae	Yes			9	5				
Russula sp. 3	Russulaceae	Yes	19	9	24	3				
Russula sp. 4	Russulaceae	Yes	54	16	3	2				
Russula sp. 5	Russulaceae	Yes	1	1						
Russula sp. 6	Russulaceae	Yes			2	1			4	1
Russula sp. 7	Russulaceae	Yes							9	4
Russula sp. 8	Russulaceae	Yes	3	1						

Macrofungal taxa	Family	Mycorrhizal	Sampling plots							
			BS		M		TN		VP	
			n1	n2	n1	n2	n1	n2	n1	n2
Russula sp. 9	Russulaceae	Yes							3	3
Russula sp. 10	Russulaceae	Yes			12	1				
Russula sp. 11	Russulaceae	Yes	5	2	6	2				
Russula sp. 12	Russulaceae	Yes	6	3						
Russula sp. 13	Russulaceae	Yes	14	1	1	1				
Russula sp. 14	Russulaceae	Yes			9	4				
Russula sp. 15	Russulaceae	Yes			1	1				
Russula sp. 16	Russulaceae	Yes			3	2				
Russula sp. 17	Russulaceae	Yes			14	3				
Russula sp. 18	Russulaceae	Yes			2	1				
Scleroderma cepa Pers.	Sclerodermataceae	Yes			2	2				
Scleroderma verrucosum (Bull.) Pers.	Sclerodermataceae	Yes							12	6
Suillus bellini (Inzenga) Watling	Suillaceae	Yes	7	3						
Trametes versicolor (L.) Lloyd	Polyporaceae	No/sap			1	1			5	5
Tricholoma portentosum (Fr.) Quél.	Tricholomataceae	Yes			2	1				
Tricholoma saponaceum (Fr.:Fr.) P.Kumm.	Tricholomataceae	Yes			1	1				
Tricholoma sejunctum (Sowerby) Quél.	Tricholomataceae	Yes			15	5				
Tricholoma squarrulosum Bres.	Tricholomataceae	Yes	4	2						
Tricholoma sulfureum (Bull.: Fr.) P. Kumm.	Tricholomataceae	Yes	37	3						
Tricholoma ustale (Fr.: Fr.) P. Kumm.	Tricholomataceae	Yes	1	1						
Vascellum pratense (Pers.) Kreisel	Lycoperdaceae	No/sap	1	1			1	1		
Xerocomus chrysenteron (Bull.) Quél.	Boletaceae	Yes	6	4					4	3
Xerocomus subtomentosus (L.: Fr.) Quél.	Boletaceae	Yes	5	3	2	2				
Species 4	Species 4	No/sap					2	1		