Universidade de Évora

# Factores ambientais que afectam a riqueza específica de macrofungos em montados de azinho – Implicações para a Gestão e Conservação

Rogério Filipe Agostinho Louro

Dissertação apresentada para a obtenção do grau de mestre em Biologia da Conservação

Orientador: Prof.ª Dr.ª Celeste Santos-Silva

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# ÍNDICE

**Factores ambientais que afectam a riqueza específica de macrofungos em montados de azinho – Implicações para a Gestão e Conservação**


## RESUMO

A riqueza específica de macrofungos e os mecanismos naturais que a influenciam são ainda pouco conhecidos. Foram utilizados modelos de regressão linear para inferir a relação existente entre a riqueza de macrofungos e diversas variáveis ambientais. De Novembro de 2005 a Abril de 2007 foram amostradas mensalmente as espécies de macrofungos presentes em montados de azinho (*Quercus rotundifolia* Lam.) no Parque de Natureza de Noudar (Alentejo, Portugal). Verificou-se que a riqueza de espécies micorrízicas aumenta com a classe etária das árvores e com a percentagem de coberto arbustivo, enquanto a riqueza de espécies sapróbias aumenta com as percentagens de cobertos arbustivo e herbáceo, sendo o coberto arbustivo relevante para ambos os grupos tróficos. Pelo exposto anteriormente, propomos que sejam mantidas faixas e pequenas manchas de vegetação arbustiva natural e que o controlo de matos seja efectuado de forma a manter intocada a rizosfera, maximizando desta forma a riqueza de macrofungos.


***Palavras-chave:*** riqueza específica de macrofungos, regressão linear, percentagem de cobertura arbustiva, montados de azinho, Portugal.

**Environmental factors affecting macrofungal richness in holm oak stands – Implications for Management and Conservation**


## ABSTRACT

Macrofungal richness is still poorly known and the natural mechanisms enhancing macrofungal diversity still remain unclear. We used linear regression models to infer the relationship between mushroom richness and several environmental variables. Therefore, a macrofungal inventory based on fruit bodies was conducted monthly, from November 2005 to April 2007, in holm oak stands (*Quercus rotundifolia* Lam.) on the Parque de Natureza de Noudar (Alentejo, Portugal).

According to our results, mycorrhizal richness increases with tree age class and shrub cover, while saprotrophic richness increases with shrub cover and herbaceous cover. Although, mycorrhizal and saprotrophic richness models differed from each other, results seem to emphasize that shrubs are of the upmost importance for both mycorrhizal and saprotrophic macrofungi in holm oak stands. Thus we propose that strips and patches of natural shrubby vegetation should be maintained and shrub control methods should keep the rhizosphere intact in order to enhance macrofungal richness.


***Key Words:*** macrofungal richness, linear regression, shrub cover, holm oak stands, Portugal.

# INTRODUÇÃO

A necessidade de proteger e conservar a biodiversidade e os recursos naturais tem vindo a assumir um papel de destaque, uma prioridade e uma preocupação generalizada na comunidade científica, face às constantes ameaças e impactos negativos inerentes à insustentabilidade dos padrões de consumo e alterações ambientais que resultam directa ou indirectamente do aumento da população humana (RSPB 2003). Há muito que a maioria dos estudos e actividades relacionadas com a protecção da Natureza e conservação da Biodiversidade incidem quase exclusivamente sobre as comunidades faunísticas e florísticas, menosprezando a importância, riqueza e diversidade do micobiota, bem como dos factores que ameaçam as comunidades micológicas. Ditosamente, esta tendência está a inverter-se, tendo-se registado nas últimas décadas um crescente interesse pelo recurso micológico, factor que tem atraído mais investigadores para esta área e consequentemente promovido a realização de mais estudos sobre taxonomia, distribuição, biologia e ecologia dos fungos. Contudo, diversas entidades empenhadas na conservação do micobiota, de que o *European Council for the Conservation of Fungi* é exemplo, continuam a alertar para a necessidade de aumentar o conhecimento da diversidade micológica, nomeadamente através de "checklists" locais, estudos prolongados e elaboração de "Red Lists", em particular nos países onde a falta de estudos base é evidente (p. ex. Albânia, Grécia e Portugal) (Senn-Irlet et al 2007).

Importa ainda considerar a gestão do habitat como uma ferramenta essencial no delineamento de estratégias para a conservação dos recursos micológicos (Molina et al 2001). Segundo Arnold (2001), a conservação *in situ* das comunidades micológicas deverá passar em primeiro lugar pela conservação e gestão adequada dos seus habitats. No entanto, no nosso País, a inclusão do micobiota em planos de conservação está ainda longe de se tornar uma realidade.

É reconhecido o papel fulcral que os fungos desempenham ao nível do equilíbrio da cadeia trófica de vários ecossistemas, assegurando a reciclagem da matéria orgânica e disponibilizando nutrientes para as espécies vegetais existentes (sapróbios), eliminando as espécies vegetais menos saudáveis (parasitas), favorecendo o crescimento e desenvolvimento de várias espécies vegetais, na medida em que lhes fornecem nutrientes essenciais (principalmente fósforo e azoto) e água e as protegem de agentes patogénicos (micorrízicos) (Alexopoulos and Mims 1979, Smith and Read 2008). Para além do seu relevante papel ecológico, muitos desses fungos produzem estruturas reprodutoras – cogumelos – muito apreciadas pelo seu valor gastronómico e actualmente considerados como um importante recurso florestal não-lenhoso. Se no passado, a colheita de cogumelos representava uma

actividade cultural enraizada e uma valiosa fonte de rendimento apenas para as populações locais (Arnold 2008, Garibay-Orijel et al 2009), hoje em dia caberá também aos proprietários gerir sustentavelmente este recurso, como previsto no Código Florestal (MADRP 2009).

Em Portugal, o conhecimento sobre a ecologia e diversidade dos macrofungos está longe de igualar a informação existente sobre a flora e fauna com que interage e coabita. Este desconhecimento deriva, em grande medida, das particularidades do método de estudo destes organismos: precariedade das frutificações (órgão mais fiável para uma identificação segura), sazonalidade com que frutificam, complexidade no reconhecimento das espécies e na taxonomia de alguns grupos, tudo isto agravado pelo insuficiente número de micologistas (Moore et al 2001).

A necessidade de conhecer as comunidades de macrofungos, bem como os factores que as afectam positiva ou negativamente é vital, pois este grupo biológico está sujeito a uma forte pressão humana, motivada pela colheita das suas estruturas reprodutoras (cogumelos). Sabe-se que são inúmeros os factores bióticos e abióticos que influenciam a presença e frutificação dos macrofungos. Cada um desses factores revela-se mais ou menos importante dependendo das características das espécies e dos grupos tróficos a que pertencem. Os factores climáticos a par de outros agentes abióticos, nomeadamente, edáficos (tipo de solo, pH, teor de matéria orgânica) e geomorfológicos (altitude, exposição, inclinação do terreno), condicionam a presença e a produtividade dos macrofungos (Villeneuve et al 1989, Brunner et al 1992, Baar 1996, Laganà et al 1999, Kernaghan and Harper 2001, Kranabetter and Kroeger 2001, Bonet et al 2004). Vários autores defendem a existência de uma forte correlação entre as comunidades de macrofungos e a composição e estrutura das comunidades vegetais (Laganà et al 1999, Bonet et al 2004, Richard et al 2004, Kernaghan et al 2003). Outros sugerem ainda que o controlo de matos, as mobilizações do solo e o pastoreio afectam a distribuição espacial e temporal dos macrofungos (Courtecuisse 2001, Pilz and Molina 2001, Wiensczyk et al 2002). Importa pois, clarificar quais os factores que influenciam directamente as comunidades de macrofungos, de forma a promover medidas de gestão sustentável para este recurso.

***Objectivos:*** De forma a fundamentar estratégias de conservação e gestão para as comunidades de macrofungos em montados de azinho, no Sul de Portugal, pretendeu-se com o presente estudo identificar quais os factores ambientais (relacionados com a vegetação, solo e geomorfologia do terreno) que influenciam a riqueza específica de macrofungos na área do Parque de Natureza de Noudar (PNN) e averiguar a existência de relações lineares entre os factores estudados e o número de espécies de macrofungos. Esta temática foi abordada e

desenvolvida culminando na redacção do artigo "Biotic and abiotic factors affecting macrofungal richness in holm oak stands of Southern Portugal", enquadrado nesta tese por uma introdução geral e considerações finais. Adicionalmente, apresenta-se a lista das espécies consideradas no âmbito do tratamento de dados efectuado (Anexo).

***Área de Estudo:*** O elevado potencial ecológico e paisagístico do Parque de Natureza de Noudar, contando com mais de 400 espécies vegetais e uma fauna diversa e abundante (Porto 2006) motivou a sua escolha para a elaboração do presente estudo. Situado no concelho de Barrancos, distrito de Beja o PNN (FIG. 1) engloba uma área total de 994,5 ha e encontra-se delimitado a norte/noroeste pelo rio Ardila e a sul pela ribeira de Múrtega.

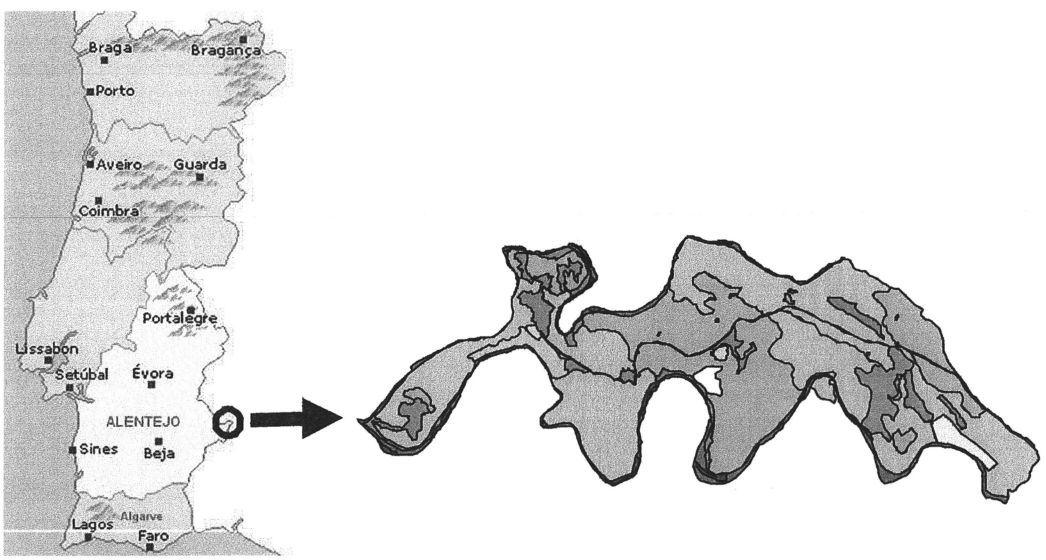


Fig. 1. Área do Parque de Natureza de Noudar e respectiva localização em Portugal

Inserido numa área classificada da Rede Natura 2000, forma um conjunto ecológico com outras áreas protegidas em Espanha, com as quais faz fronteira, nomeadamente com os Parques Naturais da Serra de Aracena e Picos de Aroche, Serra Norte e Serra de Hornachuelos. Relativamente ao enquadramento geológico, integra-se na Zona da Ossa Morena, uma das grandes unidades paleogeográficas que dividem o Maciço Ibérico, constituído por formações das Eras Pré-Câmbrica e Paleozóica. Na área do PNN predominam os "Litossolos dos climas de regime xérico", de xistos ou grauvaques, surgindo

frequentemente associados a afloramentos rochosos de xistos ou grauvaques (Piçarra et al 2001).

O PNN enquadra-se na área do macrobioclima Mediterrâneo, bioclima Pluviestacional Oceânico. A temperatura média anual do ar é de 15,8 ºC e a precipitação anual de 525,6 mm, ocorrendo quase exclusivamente na estação mais fria. O período seco (P<2T) é longo e prolonga-se geralmente de Junho a Setembro.

A espécie arbórea dominante é a azinheira (*Quercus rotundifolia* Lam.), ocupando cerca de 70 % da área total do PNN, formando povoamentos com densidades arbustivas variáveis. De acordo com Gomes (1999) os azinhais e montados enquadram-se nas formações *Pyro bourgaeana-Quercetum rotundifolia e Asparago albi-Rhamnion oleoides,* surgindo nas zonas mais degradadas formações da classe *Cisto-lavanduletea.* Nos montados abertos, são características as pastagens de trevo-subterrâneo (*Poetea bulbosae*) e as comunidades de *Tuberarietea*, sujeitas a pastoreio por gado bovino.

Num estudo previamente efectuado (Louro et al 2009) foram identificadas 162 espécies de macrofungos, para todos os biótopos presentes no PNN. Destas, foram referidas pela primeira vez 77 espécies para a região do Alentejo e 8 para Portugal. Registou-se ainda a presença de 6 espécies consideradas raras na Península Ibérica, nomeadamente *Agaricus porphyrizon* Orton, *Ileodictyon gracile* Berk., *Lactarius camphoratus* (Bull.) Fr., *Lepiota oreadiformis* Velen., *Leucoagaricus melanotrichus* var. *melanotrichus* (Malençon and Bertault) Trimbach e *Phaeomarasmius erinaceus* (Pers.) Scherff. ex Romagn.. Adicionalmente registaram-se as espécies *Amanita verna* (Bull.) Lam., *Cortinarius orellanus* Fr., *Gyroporus castaneus* (Bull.) Quél. e *Hygrocybe conica* var. *conica* (Scop.) P. Kumm.), consideradas como potencialmente ameaçadas (SMM 2008).

# Biotic and abiotic factors affecting macrofungal richness in holm oak stands of Southern Portugal

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***Abstract:*** We used linear regression models to infer the relationship between macrofungal richness and several environmental variables, in holm oak stands. Therefore a macrofungal inventory, based on fruit bodies, was conducted monthly, from November 2005 to April 2007, in pure *Quercus rotundifolia* Lam stands on the Parque de Natureza de Noudar, located in Alentejo Province, Portugal. According to our results, vegetation characteristics were the best descriptors of macrofungal richness in the studied area. Thus, mycorrhizal richness increases with tree age class and shrub cover, while saprotrophic richness increases with shrub cover and herbaceous cover. Although, mycorrhizal and saprotrophic richness models differed from each other, results seem to emphasize that shrubs are of the upmost importance for both mycorrhizal and saprotrophic macrofungi in holm oak montado ecosystems.

***Key Words:*** macrofungal richness, linear regression, shrub cover, holm oak stands, Portugal.

## INTRODUCTION

Montado ecosystems are Mediterranean savannah-like rangelands characterised by the presence of an open tree stratum (40-50 trees per ha) mainly composed of evergreen oaks (*Quercus rotundifolia* Lam or *Quercus suber* L.) (Gallardo 2003, Azul 2010). The under-canopy stratum is generally comprised of pastures and agricultural fields in a rotation scheme that includes fallows, with Mediterranean shrubs artificially kept at low densities (Carreiras et al 2006, Pereira and Fonseca 2003, Peco et al 2006, Pinto-Correia 1993). These ecosystems have resulted from a transformation process of the original cork oak and holm oak forests, by human activities, and are maintained by constant human intervention (Azul 2002). Montado ecosystems occupy extensive areas in southern Portugal and are the most common agroforestry systems in the Alentejo Province (Portugal) (DGF 2001, Azul 2010). Montados are known to simultaneously provide multiple goods and services (Vogiatzakis et al 2006) and to sustain a very high biodiversity (Plieninger and Wilbrand 2001), thus representing an

example of ecologically sustainable agroforestry system (Azul 2002).

Montado ecosystems are typically managed for three main purposes: forestry, agriculture, and extensive grazing (Pereira and Fonseca 2003). Cork removal and acorn production have been traditionally accompanied by mixed livestock raising at low stocking densities and by arable systems with long-period rotations and closed nutrient cycles, without external inputs of fodder, fertilizers and agro-chemicals (Plieninger and Wilbrand 2001). Nevertheless, socioeconomic and land use changes over the second half of the twentieth century, such as intensive grazing, mechanization of agriculture and rural desertification, resulted in soil degradation, tree scarceness, lack of regeneration and shrub encroachment that may increase considerably the risk of forest fires, pests and diseases (Ferreira 2001, ICN 2006). For instance, shrub clearing was customarily carried out manually only within selected patches, minimizing the damaged area. However, due to public forestry subsidies and motor powered machinery, shrub clearing is nowadays far more intensive, producing a more homogeneous and intense disturbance (Pérez-Ramos et al 2008).

Macrofungi are among the most important organisms in both natural and semi-natural forest ecosystems. Without saprotrophic fungi, the primary decomposers of wood and litter (Hobbie et al 1999, Robinson et al 2005, Zeller et al 2007), many nutrient cycles would be drastically altered and forest ecosystem productivities greatly reduced (Ricklefs and Miller 2000). Others, such as mycorrhizal fungi are capable of establishing symbiotic associations with the roots of most plant species, aiding in plant nutrient and water acquisition (Brunner 2001, Hartnett and Wilson 2002, Guidot et al 2003, Smith and Read 2008), altering the competitive relationships among plants of different species (Kennedy et al 2003, Egerton-Warburton et al 2007), protecting their hosts from soil pathogens (Amaranthus 1998, Hartnett and Wilson 2002, Smith and Read 2008) and from environmental extremes (Amaranthus 1998, Smith and Read 2008), and play an important role in the sequestration of C in soil (Treseder and Allen 2000). Even pathogenic fungi enhance forest diversity by killing trees that latter can become snags and logs that can be used by a variety of other organisms (O'Dell et al 1996).

However, the importance of fungi goes far beyond their fundamental role in many ecosystem functions and processes since they influence humans and various human-related activities as well (Mueller and Bills 2004). Wild mushrooms, the fruit bodies of many forest macrofungi, are nowadays regarded as one of the most important non-wood forest products (Bonet et al 2004, Garibay-Orijel et al 2009). In fact, the fruit bodies of more than 3000 macrofungal species are consumed around the world (Garibay-Orijel et al 2009), and the economic value of some of them surpasses by far the value of timber (Arnolds 1995, Honrubia 2007). Moreover,

mushrooms sudden appearance and beauty always fascinated people, making them also important in the context of nature conservation and management (Straatsma et al 2001). Most surveys dealing with macrofungi are based on fruiting bodies, since fruit bodies can be identified to species and large number of plots can be continuously monitored over a number of years (Schimt et al 1999, Dahlberg et al 1997).

The biotic and abiotic factors influencing macrofungal species occurrences and distributions are numerous and interactive (Beare et al 1995). Plant species composition is known to be an important factor shaping macrofungal communities (Bills et al 1986, Brunner et al 1992, Villeneuve et al 1989, Kernaghan and Harper 2001, Cavender-Bares et al 2009, Jumpponen et al 2010) since plants constitute both habitat and energy source for most macrofungi and many of them exhibit some degree of plant host or substratum specificity (Lodge et al 2004). Plant community's structure is also an influential factor determining the macrofungal communities present in a site (Yang et al 2006). Tree or canopy cover's influence on macrofungi has been shown in many studies, mostly in Boreal forests or conifer trees (Villeneuve et al 1989, Ruhling and Tyler 1990, Laganà et al 1999, Senn-Irlet and Bieri 1999, Jansen 1991, Bonet et al 2004, Richard et al 2004, De Bellis et al 2006). Shrub cover and herbaceous cover also have been shown to influence macrofungi occurrence and distribution (Ruhling and Tyler 1990, Richard et al 2004), as many macrofungi are known to associate with particular shrub species and even show a certain degree of host specificity (Comandini et al 2006, Eberhardt et al 2009). Plus, most *Hygrophoraceae* species are known to occur chiefly in open, grass-dominated sites (Ruhling and Tyler 1990). Stand successional changes may also have an impact on macrofungal communities (Keizer and Arnolds 1994, Senn-Irlet and Bieri 1999, Nordén and Paltto 2001, Gates et al 2005, Twieg et al 2009), through the establishment of new hosts and changes on the amount and/or quality of both litter and organic matter content (Lodge et al 2004) or simply through variations on stand photosynthetic rates and growth efficiency (Dahlberg 2002, Nara et al 2003, Bonet et al 2008). Edaphic and geomorphologic features such as soil nutrients, base saturation, organic matter, slope, aspect and altitude are also known to influence macrofungal communities present in a site (Bonet et al 2004, Engola et al 2007, Hansen 1988, Kernaghan and Harper 2001, Ruhling and Tyler 1990, Twieg et al 2009, Yang et al 2006).

The limited and often scattered knowledge on how silvicultural treatments affect fungal populations, make it extremely difficult for most forest managers to integrate mushroom species, in management programs (Martínez-Aragón et al 2007). Forest managers need a better understanding of how their choices will influence macrofungal communities and

mushroom yields if they want to explore this resource (Pilz and Molina 2001). Yet, to our knowledge only a few models regarding mushrooms have been developed so far (Yang et al 2006). For instance, Hansen (1988) used soil and litter variables to predict macrofungal occurrences in Swedish beech forests, Yang et al (2006) employed a logistic regression and a GIS expert system to model the fine-scale spatial distribution of matsutake in Yunnan, southwest China, Martínez-Aragón et al (2007) and Bonet et al (2008) developed predictive equations for ectomycorrhizal and selected edible saprotrophic fungi productivities in pine forests of the pre-Pyrenees mountains of Spain and Dahl et al (2008) used weather information to predict mushroom abundance in Norway. According to some authors, the development of mechanistic models based on empirical studies over a broad range of forest types, stand conditions, and site factors might facilitate foresters to better evaluate habitat conditions for mushrooms (Pilz et al 2001, Bonnet et al 2004, Dahl et al 2008). Moreover, land-use changes, particularly in forestry and agriculture, are the major causes of change and decline of macrofungal diversity in Europe (Senn-Irlet et al 2007). Therefore, sustaining appropriate forest habitat is essential for sustaining mushroom diversity and yields (Pilz et al 2001).

In order to find simple and applicable models that can explain how environmental condition influences macrofungal richness we developed linear regression models for wild mushroom richness in holm oak (*Quercus rotundifolia* Lam) montado ecosystem in Alentejo Province, Southern Portugal.

## MATERIAL AND METHODS

*Site description.*—The study was conducted in holm oak (*Quercus rotundifolia* Lam) stands on the Parque de Natureza de Noudar (PNN). Located near Barrancos (38° 08' N and 6° 59' W) in Alentejo Province, Portugal. The PNN encompasses a total area of 994.5 ha and it is bordered on the north-west by the Ardila River and on the south by the Múrtega stream. The climate is typically Mediterranean pluviseasonal oceanic with a mean annual air temperature of 15.8 °C and a mean annual rainfall of 525.6 mm. The dry season is usually from June to September (Mendes et al 1991). The study area is dominated by *Pyro bourgaeana - Quercetum rotundifolia* and *Asparago albi-Rhamnion oleoides* formations, although various elements of the *Cisto-lavanduletea* are rather frequent in the most degraded areas (Gomes 1999).

*Sampling and identification of macrofungi.*—Sampling was conducted monthly from

November 2005 to April 2007, in 33 permanent circular plots (250 $m^2$ each) randomly chosen in pure *Q. rotundifolia* Lam stands. All observed macrofungi epigeous sporocarps within the sampling plots were collected in order to ensure the detection of those *taxa* difficult to distinguish in the field (O' Dell et al 2004). Identification was done on-site at the moment of detection, and whenever necessary specimens were kept in a freezer at 3 °C for further verification. Representative voucher collections for all observed *taxa* were deposited at the Évora University Herbarium (UEVH- FUNGI). Each macrofungal *taxon* was included in one of the three main trophic groups: saprotrophic, parasitic or mycorrhizal, according to (Breitenbach and Kränzlin 1984, 1986, 1991, 1995, 2000, Frade and Alfonso 2003, Moreno et al 1986, Kränzlin 2005). Those *taxa* with more than one trophic group, depending on ecological and environmental conditions, were included in the most likely trophic group.

*Plot characterization.*—Environmental variables were recorded at plot level and encompassed vegetation characteristics (composition and structure), soil descriptors, slope and aspect (TABLE I). Vegetation cover was calculated separately by layer as the percentage of canopy (tree, shrub, herb or moss and lichen) projection area per plot area. To estimate tree age, annual ring counts were made on the cross sections of the core samples removed from the tree stems, at 0.5 m height with an increment borer. Tree ages were grouped into 5 classes, respectively: 10-19 years, 20-29 years, 30-39 years, 40-49 years and 50-59 years. Diameters at breast height were measured with a tree calliper at 1.30 m height. Soil samples (4 replicates per plot) were collected, using a 5 cm diameter soil probe. Soil organic matter content was calculated by weight difference after ignition (550 °C for 5 h in a muffle furnace (Nabertherm L9/C6). Soil pH measurements were done in an aqueous solution of 1:2 (earth: distilled water) using a pH meter (Metrohm 691). Soil type was consulted in Portugal soil map (SROA 1973), letters no. 44-A and 44-B, scale 1:50000, and was grouped in 5 classes ranging between 0.2 and 1. Hence 0.2 was attributed to the shallower leptosols whereas the most evolved luvisols corresponded to 1.

*Statistical Analysis.*—Environmental variables normality was assessed using Kolmogorov-Smirnov tests. Square root and logarithmic transformations were used to achieve normality when necessary. Levene's tests were employed to assess the variance homocedasticity assumption. The strength of the linear association among the dependent variables (total macrofungal richness, mycorrhizal richness and saprotrophic richness) and independent variables (TABLE I) was quantified using the Pearson product-moment correlation coefficient.

TABLE I. Environmental variables recorded at plot level, correspondent units and descriptive statistics.

| Variable | Description | Units | Mean | Median | Mode | Std. deviation | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| Ty | Tree age class | year class | 2.818 | 3.000 | 3.000 | 1.044 | 1.000 | 5.000 |
| Dbh | Mean tree diameter at breast height | meters | 0.325 | 0.340 | 0.350 | 0.093 | 0.100 | 0.600 |
| Ct | Tree cover | % | 18.393 | 16.406 | 9.375 | 8.737 | 7.813 | 35.156 |
| Cs | Shrub cover | % | 30.776 | 20.000 | 0.000 | 31.748 | 0.000 | 89.010 |
| Ch | Herbaceous cover | % | 72.576 | 90.000 | 100.000 | 30.133 | 20.000 | 100.000 |
| Ht | Mean tree height | meters | 5.456 | 5.595 | 4.927 | 0.711 | 4.140 | 6.656 |
| Hs | Mean shrub height | meters | 0.534 | 0.307 | 0.000 | 0.606 | 0.000 | 2.109 |
| Hh | Mean herbaceous height | meters | 0.201 | 0.093 | 0.062 | 0.251 | 0.017 | 1.127 |
| Sn | Shrub species number | number per plot | 3.818 | 4.000 | 3.000 | 2.455 | 0.000 | 9.000 |
| Hl | Mean litter height | meters | 0.012 | 0.010 | 0.000 | 0.012 | 0.000 | 0.040 |
| Om | Soil organic matter content | % | 8.518 | 8.200 | 7.800 | 2.273 | 4.650 | 16.350 |
| pH | Soil pH | moles per liter | 5.618 | 5.620 | 5.310 | 0.411 | 4.520 | 6.530 |
| Cml | Moss and lichen cover | % | 13.030 | 0.000 | 0.000 | 24.967 | 0.000 | 80.000 |
| As | Aspect | degrees | 213.939 | 225.000 | 360.000 | 126.527 | 30.000 | 360.000 |
| Sl | Slope | % | 11.182 | 10.000 | 2.000 | 7.896 | 2.000 | 30.000 |
| St | Soil type | soil class | 0.448 | 0.400 | 0.400 | 0.229 | 0.200 | 1.000 |

The threshold value for deciding on redundancy among independent variables was set to a significant correlation coefficient of around 0.6. Stepwise multiple linear regression analysis was then conducted to identify the set of independent variables that better explain variation in the response variables. Variable additions to the models continued until the significance level of *F* values drop below the entry value ($P < 0.05$). Among all models computed we chose the ones with the best adjustment, namely the ones with: highest adjusted coefficient of determination ($R^2$aj), negligible residuals autocorrelation (Durbin-Watson coefficient value near 2) and with a normal residuals distribution. All calculations were performed with SPSS 15.0 for Windows (SPSS Inc., Chicago, IL).

## RESULTS

Throughout the study period a total of 117 *taxa* belonging to 57 *genera* were accounted for, encompassing 79 saprotrophic and 38 mycorrhizal macrofungi. Members in the order *Agaricales* comprised 81 % of the total identified *taxa* (FIG. 2). At the genus level *Entoloma* (6 sp.), *Inocybe* (7 sp.), *Lepiota* (6 sp.), *Mycena* (7 sp.) and *Russula* (8 sp.) were the most represented genera.

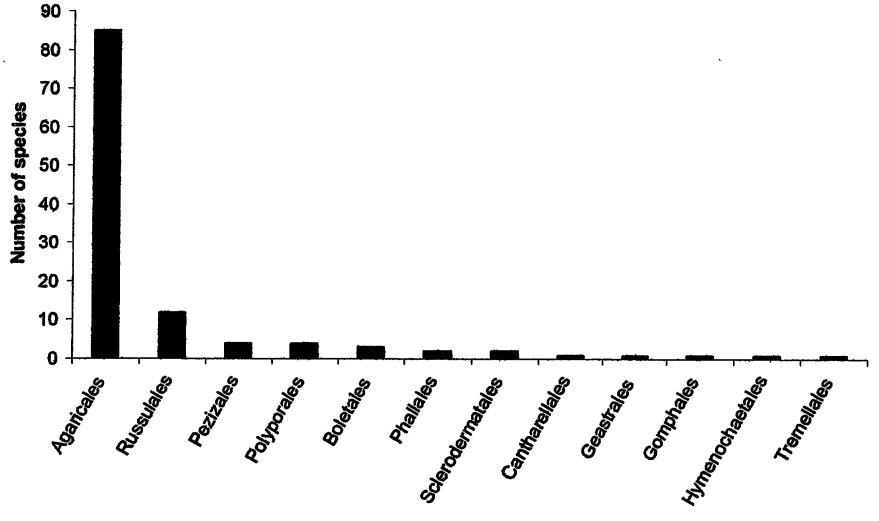


FIG. 2. Species richness of several macrofungal orders throughout the study period

Around 9 % of the total identified *taxa* showed a widespread occurrence, namely, *Astraeus hygrometricus* (Pers.) Morgan, *Gymnopus dryophilus* (Bull.) Murrill, *Laccaria laccata* (Scop.) Cooke, *Lycoperdon atropurpureum* Vittad., *Lycoperdon perlatum* Pers., *Mycena pura* (Pers.) P. Kumm., *Parasola auricoma* (Pat.) Redhead, Vilgalys and Hopple, *Psathyrella spadiceogrisea* (Schaeff.) Maire, *Psilocybe crobula* (Fr.) Singer, *Scleroderma verrucossum* (Bull.) Pers. and

*Tubaria romagnesiana* Arnolds. In contrast, 27 % of the total identified *taxa* appeared only once in one plot, during the study period.

None significant linear correlations were found between soil descriptors, slope or aspect and any dependent variable (total macrofungal richness, mycorrhizal richness and saprotrophic richness). In what regards the vegetation characteristics, shrub cover, number of shrub species, mean shrub height, herbaceous cover and litter height showed strong linear associations with some dependent variables (TABLE II). Additionally, a large number of correlations among vegetation characteristics were also found. For instance, highly significant correlations ($P<0.001$, $n=33$) were found between shrub cover and respectively, mean shrub height (0.714), shrub species number (0.776) and mean litter height (0.764).

Although the large number of correlations among stand characteristics was expected, it limited the number of variables to be used in the models due to collinearity problems. Hence, only the following variables were used in the multiple linear regression analysis: tree cover, tree age class, shrub cover, aspect, slope, soil pH and organic matter content.

TABLE II. Pearson's product-moment correlation coefficient (R-values). Dependent variables: mycorrhizal richness (Mr), saprotrophic richness (Sr), total macrofungal richness (Tr). * represent significant correlations at $P < 0.05$ and ** at $P < 0.001$.

| Independent. Variables | Tr | Mr | Sr |
|---|---|---|---|
| Tree age class | 0.395* | 0.381* | 0.149 |
| Tree cover | 0.204 | 0.090 | 0.182 |
| Shrub cover | 0.638** | 0.746** | 0.147 |
| Herbaceous cover | -0.360 | -0.678** | 0.479** |
| Mean tree height | 0.065 | -0.158 | 0.346* |
| Mean shrub height | 0.227 | 0.479* | -0.382* |
| Mean herbaceous height | -0.342 | -0.481 | 0.203 |
| Shrub species number | 0.467* | 0.608** | -0.203 |
| Mean litter height | 0.493** | 0.708** | -0.315 |
| Soil organic matter content | 0.085 | 0.243 | -0.241 |
| Soil pH | -0.115 | -0.031 | -0.133 |
| Soil humidity | -0.436 | -0.273 | -0.266 |
| Moss and lichen cover | 0.505 | 0.158 | 0.429 |
| Aspect | 0.024 | -0.097 | 0.188 |
| Slope | -0.072 | -0.045 | -0.045 |
| Soil type | -0.238 | -0.410 | 0.258 |

The best adjusted models are shown in TABLE III. Shrub cover, tree age class and herbaceous cover were the most significant explanatory variables of total macrofungal richness. For mycorrhizal richness the best set of explanatory variables was shrub cover and tree age class, whereas for saprotrophic richness was shrub cover and herbaceous cover. All regressions forcefully passed through the origin since the constant always showed a significance value higher than 0.05 in all regressions.

TABLE III. Multiple linear regression models for the analysed response variables. Ty - Tree age class, Cs - Shrub cover, Ch - Herbaceous cover

| Response Variables | Model summary | $R^2$ | P-Values |
|---|---|---|---|
| Total macrofungal richness (Tr) | Tr = 0.177Cs + 1.435Ty + 0.068Ch | 0.948 | < 0.001 |
| Mycorrhizal richness (Mr) | Mr = 0.123Cs + 0.739Ty | 0.830 | < 0.001 |
| Saprotrophic richness (Sr) | Sr = 0.058Cs + 0.096Ch | 0.929 | < 0.001 |

In order to examine exactly how the number of macrofungal species varied in regard to the explanatory variables, we plotted the response variables as a function of each independent variable, while maintaining the other factors equal to their mean (FIG. 3, A-C). According to our results shrub cover exert more influence on mycorrhizal richness than on the number of saprotrophic species. Thus, total macrofungal richness augment with shrub cover seems mainly attributable to its influence on mycorrhizal richness. In regard to tree age class, both total macrofungal and mycorrhizal richness increase linearly with tree age. Nevertheless, the former rises more steeply than the later, possibly due to a positive, but not linear, saprotrophic richness response to tree age class. Herbaceous cover, on the other hand positively influences both saprotrophic and total macrofungal richness. However, the less pronounced slope on the total macrofungal richness linear equation seems to point out a negative effect of herbaceous cover on mycorrhizal richness.

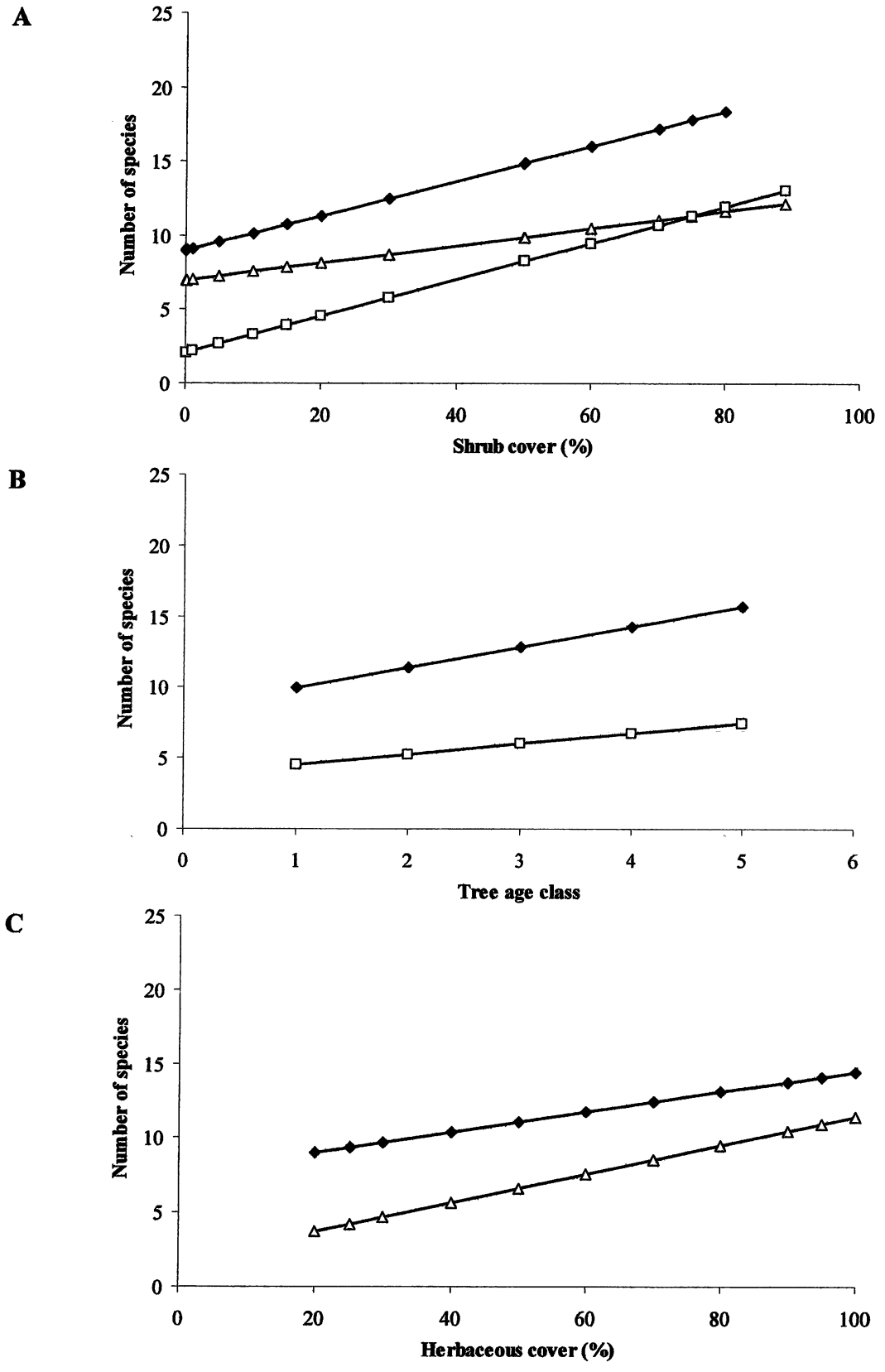


FIG. 3. Macrofungal richness (♦- total, □ - mycorrhizal, Δ - saprotrophic) as a function of shrub cover (A), tree age class (B) and herbaceous cover (C) according the equations shown on TABLE III.

## DISCUSSION

Many authors consider edaphic and geomorphological features as important factors affecting the macrofungal communities present in a site. However neither edaphic nor geomorphologic variables were linearly correlated with macrofungal richness or were included in any of the models computed. It is possible that those variables are only relevant in a wider spatial scale, and therefore our results are merely a reflection of the narrow spectre of this study, or perhaps the relationship between them and macrofungal richness is other than linear. On the contrary vegetation descriptors seemed to suitably explain the variation in the macrofungal richness of the studied area.

Results showed that macrofungal richness was strongly related to both tree age and under-canopy vegetation cover and that the number of macrofungal species increases linearly with them. Yet, total macrofungal, mycorrhizal and saprotrophic richness models differ form each other.

Concerning mycorrhizal richness, results showed that it only increases linearly with tree age and with the percentage of shrub cover. Concerning tree age, recent works propose that the successional changes effects on stand photosynthetic rates and growth efficiency may explain the variation on the abundance of ectomycorrhizas and their fruit body production (Dahlberg 2002, Nara et al 2003, Bonet et al 2008). As for shrub cover's influence on mycorrhizal richness, Richard et al (2004) working in an old-growth Mediterranean forest dominated by *Quercus ilex* L., found both ectomycorrhizal and saprotrophic richness positively correlated to *Arbutus unedo* L. density. Also, Comandini et al (2006) recognized more than 200 fungal species to be associated with *Cistus* spp. (the most abundant shrub *taxon* in our study area), which points out the importance of shrubs as potential hosts for macrofungi in Mediterranean forest ecosystems. Furthermore, according to some authors, microenvironmental conditions such as light, moisture and temperature encompassed by denser canopy cover can enhance mycorrhizal diversity (Kranabetter and Kroeger 2001, Bonnet et al 2004, Santos-Silva et al 2010). In a similar way, we propose that is also plausible that microenvironmental conditions under shrubby vegetation may allow the establishment of those mycorrhizal species that prefer shadier areas, especially in cases where tree density is low.

Saprotrophic richness increases linearly with shrub and herbaceous cover. Resource availability is probably the more likely explanation for the linear relationship between saprotrophic richness and shrub cover. Saprotrophic fungi are intimately associated to the substrate from which they feed, and many show preference for a specific shrub litter (Roberts et al 2004). Moreover, the

amount and quality of available plant litter are known to alter the composition and structure of decomposer communities (Wardle and Lavelle 1997, Wardle et al 2006). Thus, it seems possible that shrub contribution to the amount and quality of soil litter in our plots may have favoured saprotrophic richness as well.

As for herbaceous cover, Maggi et al (2005) found that it can affect microenvironmental conditions at soil surface, providing shade, increasing soil moisture content and thus providing a greater protection from heat. This may explain the increase in saprotrophic richness, as microenvironmental conditions are believed to directly impact the fruitbodies transpiration rates (Kernaghan and Harper 2001) and consequently constrain mushroom production. Substrate specificity also can explain some of the variation in saprotrophic richness. In fact, several hundred saprotrophic basidiomycete fungi are more often found in grasslands (Griffith and Roderick 2008), or are known to occur mostly in open, grass-dominated sites (Ruhling and Tyler 1990). Additionally, cattle's presence is frequent associated to open areas where herbaceous cover dominates. This probably enables the occurrence of many coprophilous or subcoprophilous macrofungi, usually linked to herbivorous dung.

In summary, with this study we aimed to develop simple and accurate macrofungal richness models to aid nature conservation managers and foresters to better understand how their decisions can affect macrofungal richness. Our results are therefore encouraging because they demonstrate that macrofungal richness variations can be fairly explained by vegetation characteristics that can be readily influenced by silvicultural interventions. We also show that shrub cover is probably of the upmost importance for macrofungal richness. If we consider that in this ecosystem shrubs are usually cleared out or artificially kept at low densities to prevent the risk of forest fires, new strategies may have to be devised in order to successfully manage macrofungal richness. This work is a contribution to the knowledge of fungal ecology in holm oak montado ecosystems and further studies in other forest types with different stand characteristics are still necessary in order to fully understand the natural mechanisms affecting macrofungal richness.

## ACKNOWLEDGEMENTS

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# CONSIDERAÇÕES FINAIS

O Parque de Natureza de Noudar alberga uma importante comunidade de macrofungos associada ao montado de azinho que importa conservar e gerir de forma sustentável. No presente estudo, as relações lineares observadas entre a riqueza específica de macrofungos e descritores da vegetação permitiram a elaboração de modelos simples e aplicáveis, que poderão constituir uma ferramenta útil para a elaboração de planos de gestão e conservação da natureza, ou para a elaboração de planos de gestão florestal que visem uma abordagem multifuncional. Verificou-se que o sub-coberto arbustivo afecta directamente o número de espécies de macrofungos. Este facto é extremamente importante, pois nos sistemas de montado a gestão do sub-coberto é uma das intervenções silvoculturais mais frequentes e necessária. Se por um lado, é necessário controlar o coberto arbustivo de forma a minimizar os riscos de incêndio e permitir a facilidade das actividades relacionadas com os aproveitamentos lenhosos, a existência do coberto arbustivo é imperiosa para a protecção do solo, abrigo da fauna e, de acordo com os nossos resultados, para as comunidades de macrofungos. Desta forma, a gestão equilibrada do coberto arbustivo passa por manter faixas e/ou áreas onde a vegetação arbustiva original seja intocada e efectuar o controlo de matos de forma a minimizar perturbações no solo, p. ex. recorrendo a corta-matos, e simultaneamente reduzir a carga combustível. Adicionalmente, quando se pretende seleccionar áreas com interesse para a conservação de macrofungos é igualmente necessário atender à idade dos povoamentos florestais e à cobertura herbácea, dado que a riqueza específica de macrofungos depende também destes factores.

Este trabalho é uma contribuição para o conhecimento da ecologia dos macrofungos em ecossistemas de montado de azinho. Contudo, novos estudos em povoamentos com características diversas das estudadas são ainda necessários a fim de compreender totalmente os mecanismos que influenciam a riqueza específica de macrofungos.

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# ANEXO

Listagem das espécies de macrofungos assinaladas em montados de azinho (povoamentos puros) no Parque de Natureza de Noudar, durante o período de amostragem, e respectivos grupos tróficos.

| Espécie | Grupo trófico |
|---|---|
| *Agaricus arvensis* Schaeff. | Sapróbio |
| *Agaricus porphyrizon* P.D. Orton | Sapróbio |
| *Agrocybe pediades* (Fr.) Fayod | Sapróbio |
| *Amanita ceciliae* (Berk. & Broome) Bas | Micorrízico |
| *Amanita ponderosa* Malençon & R. Heim | Micorrízico |
| *Amanita rubescens* Pers. | Micorrízico |
| *Amanita vaginata* (Bull.) Lam. | Micorrízico |
| *Astraeus hygrometricus* (Pers.) Morgan | Micorrízico |
| *Boletus chrysenteron* Bull. | Micorrízico |
| *Bovista delicata* Berk. & M.A. Curtis | Sapróbio |
| *Bovista dermoxantha* (Vittad.) De Toni | Sapróbio |
| *Clavariadelphus pistillaris* (L.) Donk | Sapróbio |
| *Clavulina cinerea* (Bull.) J. Schröt. | Sapróbio |
| *Clitocybe costata* Kühner & Romagn. | Sapróbio |
| *Clitocybe font-queri* R. Heim | Sapróbio |
| *Clitocybe obsoleta* (Batsch) Quél. | Sapróbio |
| *Clitocybe squamulosoides* Bon | Sapróbio |
| *Coprinus alopecius* Lasch | Sapróbio |
| *Cortinarius anomalus* (Fr.) Fr. | Micorrízico |
| *Cortinarius bulliardii* (Pers.) Fr. | Micorrízico |
| *Cortinarius orellanus* Fr. | Micorrízico |
| *Crucibulum leave* (Huds.) Kambly | Sapróbio |
| *Cyathus olla* (Batsch.) Pers | Sapróbio |
| *Cyathus stercoreus* (Schwein.) De Toni | Sapróbio |
| *Cystoderma amianthinum* (Scop.) Fayod | Sapróbio |
| *Entoloma cistophilum* Trimbach | Sapróbio |
| *Entoloma clypeatum* (L.) P. Kumm. | Sapróbio |
| *Entoloma hebes* (Romagn.) Trimbach | Sapróbio |
| *Entoloma occultopigmentatum* Noordel. & Arnolds | Sapróbio |
| *Entoloma papillatum* (Bres.) Dennis | Sapróbio |
| *Entoloma undatum* (Fr.) M.M. Moser | Sapróbio |
| *Galerina vittiformis* (Fr.) Earle | Sapróbio |
| *Geastrum elegans* Vittad. | Sapróbio |
| *Gymnopus dryophilus* (Bull.) Murrill | Sapróbio |
| *Gymnopus impudicus* (Fr.) Antonín, Halling & Noordel. | Sapróbio |
| *Gyroporus castaneus* (Bull.) Quél. | Micorrízico |
| *Hebeloma cistophilum* Maire | Micorrízico |
| *Hebeloma mesophaeum* (Pers.) Quél | Micorrízico |
| *Helvella lacunosa* Afzel. | Sapróbio |
| *Helvella leucomelaena* (Pers.) Nannf. | Sapróbio |
| *Hydropus floccipes* (Fr.) Singer | Sapróbio |
| *Hygrocybe conica* (Scop.) P. Kummer | Sapróbio |
| *Hygrocybe miniata* (Fr.) P. Kumm. | Sapróbio |
| *Hygrocybe russocoriacea* (Berk. & Jos.K. Mill.) P.D. Orton & Watling | Sapróbio |
| *Hygrocybe virginea* (Wulfen) P. D. Orton & Watling | Sapróbio |
| *Hygrophorus arbustivus* Fr. | Micorrízico |
| *Hygrophorus eburneus* (Bull.) Fr. | Micorrízico |

| Espécie | Grupo trófico |
|---|---|
| *Ileodictyon gracile* Berk. | Sapróbio |
| *Inocybe asterospora* Quél. | Micorrízico |
| *Inocybe bongardii* (Weinm.) Quél. | Micorrízico |
| *Inocybe calospora* Quél. | Micorrízico |
| *Inocybe flocculosa* (Berk.) Sacc. | Micorrízico |
| *Inocybe fraudans* (Britzelm.) Sacc. | Micorrízico |
| *Inocybe geophylla* (Pers.) P. Kumm. | Micorrízico |
| *Inocybe praetervisa* Quél. | Micorrízico |
| *Laccaria laccata* (Scop.) Cooke | Micorrízico |
| *Lactarius camphoratus* (Bull.) Fr. | Micorrízico |
| *Lactarius chrysorrheus* Fr. | Micorrízico |
| *Lactarius cistophilus* Bom & Trimbach | Micorrízico |
| *Lactarius lacunarum* Romagn. ex Hora | Micorrízico |
| *Leccinum corsicum* (Rolland) Singer | Micorrízico |
| *Lepiota clypeolaria* (Bull.) P. Kumm | Sapróbio |
| *Lepiota griseovirens* Maire | Sapróbio |
| *Lepiota oreadiformis* Velen. | Sapróbio |
| *Lepiota pseudolilacea* Huijsman | Sapróbio |
| *Lepiota subgracilis* Wasser | Sapróbio |
| *Lepista nuda* (Bull.) Cooke | Sapróbio |
| *Lepista sordida* (Fr.) Singer | Sapróbio |
| *Leucoagaricus melanotrichus* (Malençon & Bertault) Trimbach | Sapróbio |
| *Lycoperdon atropurpureum* Vittad. | Sapróbio |
| *Lycoperdon excipuliforme* (Scop.) Pers. | Sapróbio |
| *Lycoperdon perlatum* Pers. | Sapróbio |
| *Lycoperdon pratense* Pers. | Sapróbio |
| *Marasmius torquescens* Quél. | Sapróbio |
| *Melanoleuca melaleuca* (Pers.) Murrill | Sapróbio |
| *Mycena aetites* (Fr.) Quél. | Sapróbio |
| *Mycena erubescens* Höhn | Sapróbio |
| *Mycena filopes* (Bull.) P. Kumm | Sapróbio |
| *Mycena flavescens* Velen. | Sapróbio |
| *Mycena galopus* (Pers.) P.Kumm | Sapróbio |
| *Mycena pura* (Pers.) P. Kumm. | Sapróbio |
| *Mycena sanguinolenta* (Alb. & Schwein.) P. Kumm. | Sapróbio |
| *Omphalina pyxidata* (Bull.) Quél. | Sapróbio |
| *Panaeolina foenisecii* (Pers.) Maire | Sapróbio |
| *Panaeolus sphinctrinus* (Fr.) Quél. | Sapróbio |
| *Parasola auricoma* (Pat.) Redhead, Vilgalys & Hopple | Sapróbio |
| *Peziza succosa* Berk. | Sapróbio |
| *Phallus impudicus* L. | Sapróbio |
| *Pholiota highlandensis* (Peck) A.H. Sm. & Hesler | Sapróbio |
| *Pluteus phlebophorus* Cooke | Sapróbio |
| *Pluteus podospileus* Sacc. & Cub | Sapróbio |
| *Polyporus alveolaris* (D.C.) Bondartsev & Singer | Sapróbio |
| *Polyporus arcularius* (Batsch.) Fr. | Sapróbio |
| *Polyporus meridionalis* (A. David) H. Jahn | Sapróbio |
| *Psathyrella conopilus* (Fr.) A. Pearson & Dennis | Sapróbio |
| *Psathyrella panaeoloides* (Maire) M.M. Moser | Sapróbio |
| *Psathyrella spadiceogrisea* (Schaeff.) Maire | Sapróbio |
| *Psilocybe coprophila* (Bull.) P. Kumm | Sapróbio |
| *Psilocybe crobula* (Fr.) Singer | Sapróbio |
| *Rhodocybe nitellina* (Fr.) Singer | Sapróbio |
| *Rickenella fibula* (Bull.) Raithelh | Sapróbio |

| Espécie | Grupo trófico |
|---|---|
| *Russula amoenolens* Romagn. | Micorrízico |
| *Russula atropurpurea* (Krombh.) Britzelm. | Micorrízico |
| *Russula cyanoxantha* (Schaeff.) Fr. | Micorrízico |
| *Russula delica* Fr. | Micorrízico |
| *Russula graveolens* Romell | Micorrízico |
| *Russula nigricans* (Bull.) Fr. | Micorrízico |
| *Russula pectinatoides* Peck | Micorrízico |
| *Russula pseudo-olivascens* Kärcher | Micorrízico |
| *Scleroderma verrucosum* (Bull.) Pers. | Micorrízico |
| *Tarzetta catinus* (Holmsk.) Korf & J.K. Rogers | Sapróbio |
| *Trametes versicolor* (L.) Lloyd | Sapróbio |
| *Tremella foliacea* Pers. | Sapróbio |
| *Tricholoma saponaceum* (Fr.) P. Kumm. | Micorrízico |
| *Tricholoma squarrulosum* Bres. | Micorrízico |
| *Tubaria romagnesiana* Arnolds | Sapróbio |
| *Volvariella gloiocephala* (DC.) Boekhout & Enderle | Sapróbio |