Universidade de Évora

Departamento de Biologia

MESTRADO EM BIOLOGIA DA CONSERVAÇÃO

RIQUEZA ESPECÍFICA E PRODUTIVIDADE DE CARPÓFOROS DE FUNGOS EPÍGEOS EM MONTADO DE SOBREIRO (*QUERCUS SUBER* L.) NO SUL DE PORTUGAL – RELAÇÃO COM FACTORES MICROAMBIENTAIS

MARIA DA LUZ JEREMIAS CARDINHA DO MAIO CALADO

DISSERTAÇÃO ORIENTADA PELA PROFESSORA DOUTORA CELESTE SANTOS E SILVA

Esta dissertação inclui as críticas e sugestões feitas pelo júri.

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RESUMO

Pretendeu-se com o presente estudo caracterizar a comunidade de macrofungos num ecossistema de montado de sobro (*Quercus suber* L.) e avaliar as condições microambientais que favorecem a sua presença e frutificação. Seleccionaram-se quatro parcelas de amostragem com diferentes estratégias de gestão, em relação à estratificação do coberto vegetal e à intensidade de pastoreio. A amostragem de carpóforos realizou-se durante um período de frutificação (2005-2006).

A riqueza específica de macrofungos (59 espécies) e a dominância das espécies sapróbias (76 %) resultaram, provavelmente, da interacção entre as condições climáticas e a configuração espacial deste sistema. A comparação entre as parcelas evidenciou a importância das particularidades microambientais na presença e abundância de diferentes grupos tróficos. Os resultados permitiram definir estratégias de gestão do ecossistema, numa perspectiva sustentável, que irão favorecer as espécies micorrízicas, assegurando, simultaneamente, a protecção e sanidade dos hospedeiros, a maximização da qualidade dos seus sub-produtos e a provável frutificação de cogumelos comestíveis, que constituirá uma mais-valia adicional.

PALAVRAS-CHAVE

Macrofungos, montado, produtividade, riqueza específica, sustentabilidade

ABSTRACT

SPECIES RICHNESS AND PRODUCTIVITY OF EPIGEOUS FUNGAL SPOROCARPS IN A CORK OAK (*QUERCUS SUBER* L.) STAND IN SOUTHERN PORTUGAL – RELATION WITH MICRO-ENVIRONMENTAL FACTORS

The aim of the present study was the characterization of macro-fungal communities in a cork oak (*Quercus suber* L.) *montado* (open forest) ecosystem and to evaluate micro-environmental conditions that favour occurrence and fructification of those communities. Four sampling plots were selected with different management strategies, in terms of system stratification and livestock pressure. Sporocarp sampling was performed during one fructification period (2005-2006).

Species richness (59 macrofungi) and the dominance of saprobic species (76 %) may be related to unfavourable conditions resulting from interaction between climatic conditions and the spatial configuration of this ecosystem.

Comparison between sampling plots showed distinct proportions of trophic groups, suggesting that particular micro-environmental conditions influence colonization and fructification of macro-fungal communities.

Sustainable ecosystem management should focus more on favouring ECM species, assuring simultaneously host protection and health, the maximization of sub-products quality (cork and acorn) and the probable fructification of edible sporocarps with high economic and gastronomic value.

KEY WORDS

Macrofungi, cork oak, productivity, species richeness, sustainability

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I. Introdução Geral

- O QUE SÃO, CARACTERÍSTICAS PRINCIPAIS

Os fungos surgem em quase todos os ecossistemas, terrestres ou aquáticos (Rodríguez *et al.* 2004), estando a sua presença, a maior parte das vezes, quase imperceptível.

Actualmente desconhece-se o número total de espécies de fungos existentes em todo o mundo e as estimativas mais recentes sugerem cerca de 1,5 milhões (Hawksworth 1991; Hawksworth 2001).

Os fungos representam um grupo de organismos com características particulares e, por isso, estão incluídos num Reino distinto – *Fungi*. Com algumas excepções, os fungos possuem corpos filamentosos e multicelulares, limitados por paredes celulares, não se deslocam e reproduzem-se assexuada e sexuadamente por esporos (Alexopoulos & Mims 1979; Kirk *et al.* 2001; Blackwell & Spatafora 2004). Por outro lado, são desprovidos de clorofila e, consequentemente, não têm capacidade de sintetizar o próprio alimento (heterotróficos), dependendo dos compostos orgânicos complexos sintetizados por outros organismos (Hudson 1980); de uma maneira geral, a digestão é extracelular, pela secreção e acção de enzimas, sendo as substâncias mais simples absorvidas para o interior das células do fungo, através das paredes e membranas celulares (Rodríguez *et al.* 2004).

As estruturas somáticas, com poucas excepções, exibem pouca diferenciação e praticamente não apresentam divisão de funções. Estas estruturas, que constituem o corpo do fungo, representam filamentos que se alongam por crescimento apical, ramificam e distribuem no substrato. Cada filamento denomina-se hifa e é constituído por uma parede fina, transparente, tubular, preenchida ou delineada com uma camada de protoplasma, que varia em espessura. A composição química da parede celular dos fungos não é semelhante para todos os fungos e para a mesma espécie, podendo variar em determinadas circunstâncias ou por factores ambientais externos (Alexopoulos & Mims 1979). Cada filamento encerra numerosos núcleos, compartimentados em septos ou não.

O conjunto das hifas denomina-se micélio, que, em alguns fungos, constitui cadeias longas e espessas. Durante determinados estádios do ciclo de vida da maioria dos fungos, o micélio organiza-se em falsos tecidos (plectênquima, prosênquima e pseudoparênquima). O prosênquima e pseudoparênquima encontram-se em vários tipos de estruturas somáticas (estroma e esclerócio) e reprodutivas formadas pelos fungos (Alexopoulos & Mims 1979).

O micélio surge, geralmente, como um pequeno tubo de germinação que emerge de um esporo. O micélio tende a estender-se em todas as direcções, mais ou menos equitativamente, a partir de um ponto central, constituindo uma colónia mais circular que esférica. As hifas dos fungos têm a possibilidade de crescer indefinidamente em condições favoráveis.

A maioria dos fungos tem a capacidade de sintetizar as proteínas essenciais ao seu crescimento, utilizando os hidratos de carbono (preferencialmente glicose ou maltose) de que depende para a obtenção de energia e fontes de azoto orgânico e inorgânico e vários elementos minerais (C, O, H, N, P, K, Mg, S, B, Mn, Cu, Mo, Fe e Zn) (Alexopoulos & Mims 1979).

- REPRODUÇÃO

No que respeita à reprodução, pode ser assexuada ou sexuada. Na formação das estruturas reprodutivas, sexuais ou assexuais, todo o organismo poderá estar envolvido ou somente uma pequena porção do mesmo (Alexopoulos & Mims 1979).

A reprodução assexuada (somática ou vegetativa) não envolve a união de núcleos, células ou órgãos sexuais. Este tipo de reprodução inclui qualquer tipo de desenvolvimento de novos indivíduos: divisão de um organismo unicelular em células-filhas; fragmentação de um organismo multicelular em fracções independentes, que originam novos indivíduos; gemiparidade de células somáticas ou esporos, em que cada gema produz um novo indivíduo; produção de esporos, em que cada esporo germina e contribui para a formação do micélio (Alexopoulos & Mims 1979). Em geral, a reprodução assexuada é mais importante para a propagação da espécie, uma vez que resulta na produção de numerosos indivíduos ao longo de diversos ciclos repetidos durante o período de reprodução (Alexopoulos & Mims 1979).

A reprodução sexuada envolve a união de dois núcleos compatíveis (Alexopoulos & Mims 1979), contribuindo para a variabilidade genética e consequente sucesso da espécie.

Este tipo de reprodução ocorre quando se cruzam 2 hifas haplóides e desencadeia-se em 3 etapas sequenciais, durante momentos específicos do ciclo de vida, especificamente, a plasmogamia (reúne dois núcleos haplóides na mesma célula), cariogamia (une os dois núcleos num diplóide) e a meiose (origina quatro núcleos haplóides) (Alexopoulos & Mims 1979). Os esporos, quando maduros, são lançados para o exterior e, em condições favoráveis, germinam e originam hifas haplóides (Rodríguez *et al.* 2004).

As estruturas reprodutivas diferenciam-se das estruturas somáticas e exibem uma variedade de formas, que constituem a base de classificação dos fungos (Alexopoulos & Mims 1979).

- PRINCIPAIS GRUPOS

A classificação dos fungos tem sido objecto de discórdia e de constantes alterações ao longo dos tempos (Blackwell & Spatafora 2004). As diferenças da classificação resultam de diferenças de interpretação dos dados, ainda incompletos, da estrutura, desenvolvimento e fisiologia dos fungos (Alexopoulos & Mims 1979).

Os estudos que incidiram na filogenia dos fungos reforçaram a teoria da origem polifilética de muitos grupos, o que significa que as morfologias seriam convergentes, tendo derivado de diversas linhas independentes de eucariotas. A maioria destes estudos filogenéticos considera, somente, os caracteres moleculares dos genes do RNA ribossomal (rDNA). Actualmente, a verificação e comparação de outras regiões do DNA, que não o rDNA, e a valorização de caracteres fenotípicos na interpretação filogenética dos grupos, veio solucionar conflitos originados pela construção de cladogramas baseados simplesmente em sequências genéticas (Blackwell & Spatafora 2004).

Historicamente, o Reino *Fungi* englobava todos os organismos eucarióticos, multicelulares, heterotróficos por absorção, não incluídos no Reino Animal *sensu lato*. Apesar de alguma discórdia, Kirk *et al.* (2001) refere quatro Divisões: *Chytridiomycota, Zygomycota, Basidiomycota e Ascomycota*. Os *Ascomycota* e *Basidiomycota* constituem as duas maiores Divisões do Reino *Fungi* e, conjuntamente, incluem mais de 95% de todos os *taxa* de fungos conhecidos (Kirk *et al.* 2001).

A Divisão *Basidiomycota* inclui as espécies que produzem esporos em estruturas particulares denominadas basídios. Os basídios são células onde ocorre a cariogamia e meiose e, consequentemente, onde se formam os esporos haplóides - basidiósporos. Existe uma grande variação na morfologia do basídio, no número de esporos formados e como os esporos surgem na superfície do basídio. Tipicamente, formam-se 4 esporos em cada basídio, que surgem no ápice, suportados em esterigmas (Alexopoulos & Mims 1979; Kirk *et al.* 2001).

Os *Basidiomycota* subdividem-se em três classes, *Basidiomycetes*, *Ustilaginomycetes* e *Urediniomycetes* (Kirk *et al.* 2001). Para além da presença de basídios, a maioria das espécies possui balistósporos e ansas de anastomose. Os balistósporos representam esporos sexuais ou assexuais, produzidos por basídios, hifas ou células de levedura, que são lançados para o ar, por um mecanismo de

"catapulta" devido à tensão superficial. Este mecanismo é activado quando uma gota de líquido presente na base do esporo, próximo à inserção no esterigma, se funde com uma película de líquido na superfície do esporo. Esta rápida coalescência dos líquidos conduz a uma alteração do centro de massa do esporo, contribuindo para a projecção explosiva (Alexopoulos & Mims 1979; Kirk *et al.* 2001).

As ansas de anastomose representam um prolongamento externo das hifas que se forma quando as células apicais das hifas dicarióticas se dividem (Alexopoulos & Mims 1979; Kirk *et al.* 2001).

A maioria dos *Basidiomycota* produz basídios em estruturas multicelulares reprodutivas, denominadas corpos de frutificação, esporocarpos, carpóforos ou, mais vulgarmente, cogumelos (Kirk *et al.* 2001). Os carpóforos poderão ser aéreos ou subterrâneos e assumir uma variedade considerável de formas, tamanhos, cores, texturas e particularidades mais ou menos complexas (Courtecuisse & Duhem 1995; Wiensczyk *et al.* 2002).

- Modos de nutrição, ecologia e importância geral dos fungos

Em consequência da sua limitação adaptativa, no que respeita ao modo de nutrição, os fungos adoptaram diferentes estratégias para subsistirem, adquirindo o carbono que necessitam a partir de outros seres vivos, de matéria orgânica ou através do estabelecimento de relações simbiose com organismos autotróficos (Rodríguez *et al.* 2004).

A estratégia de nutrição dos fungos atribui-lhes uma importância fulcral no equilíbrio do ecossistema e nas actividades relacionadas com o Homem (Mueller & Bills 2004).

Os fungos têm sido utilizados em actividades humanas, como no fabrico de cerveja, outro tipo de fermentação industrial e nas indústrias biotecnológicas e farmacêuticas (Alexopoulos & Mims 1979; Mueller & Bills 2004).

Ao nível do ecossistema, destacam-se, pela sua importância, os três grupos funcionais - fungos parasitas, sapróbios e micorrízicos.

Os fungos parasitas dependem de um hospedeiro vivo para subsistirem e estabelecem uma relação prejudicial para o hospedeiro. No entanto, estes fungos assumem um papel importante na manutenção da fitossanidade do sistema, através da eliminação dos indivíduos menos saudáveis.

Os fungos sapróbios colonizam restos vegetais ou animais ou absorvem material orgânico, exsudado ou libertado de organismos mortos ou vivos (Hudson 1980), assegurando a reciclagem da matéria

orgânica, a disponibilização de nutrientes para as espécies vegetais existentes e a libertação de dióxido de carbono essencial na fotossíntese (Alexopoulos & Mims 1979).

Os fungos micorrízicos estabelecem relações de simbiose com espécies arbóreas, arbustivas ou herbáceas, adquirindo uma parte dos produtos fotossintetizados produzidos pelo hospedeiro. Em compensação, os fungos favorecem o crescimento e desenvolvimento dos hospedeiros, na medida em que melhoram a eficiência de captação de nutrientes essenciais (principalmente fósforo e azoto) e água, protegem a planta de agentes patogénicos, da acção de metais pesados, condições de escassez hídrica e oscilações extremas de pH e temperatura, permitem a agregação das partículas de solo constituindo uma estrutura edáfica favorável, facilitam a transferência subterrânea de nutrientes entre plantas e alteram as relações competitivas interespecíficas (Smith & Read 1997).

A associação micorrízica resulta, fundamentalmente, de uma estratégia para resistir aos constrangimentos ambientais dentro de um nicho ecológico, adoptada, principalmente, pelas plantas vasculares. De facto, cerca de 80% das plantas vasculares formam micorrizas (Wang & Qiu 2006).

Esta relação fungo-hospedeiro ocorre ao nível radicular, em que o fungo poderá envolver as raízes (fungos ectomicorrízicos) ou penetrar no interior da parede celular das raízes das plantas (fungos endomicorrízicos) (Wang & Qiu 2006).

Apesar da associação das plantas vasculares com fungos endomicorrízicos ser a mais ancestral e difundida, existe uma grande diversidade de fungos ectomicorrízicos (ECM), pertencentes à Divisão *Ascomycota* e *Basidiomycota*, que se estabelecem importantes relações simbióticas com o hospedeiro (Wang & Qiu 2006).

Os fungos ECM apresentam uma variedade notável de morfotipos (Valentine *et al.* 2004) e variações fisiológicas inter e intra-específicas, que representam adaptações aos diferentes ecótopos face às pressões ambientais em diferentes regiões geográficas, ou, numa escala mais pequena, à heterogeneidade das condições edáficas (Cairney 1999). Paralelamente exibem diferentes graus de especificidade para os grupos de plantas hospedeiras (Molina *et al.* 1992). Os fungos ECM que se associam a múltiplos hospedeiros poderão translocar nutrientes entre as diversas espécies de plantas (Simard *et al.* 1997) e, desta forma, a competição pelos nutrientes do solo entre as plantas poderá resultar das interacções individuais com o simbionte comum (Horton & Bruns 1998). Contrariamente, um fungo que estabelece uma relação mais estreita com os hospedeiros (associação a um só género), em resultado de adaptações fisiológicas aos produtos químicos produzidos por estes, poderá assegurar, aos seus hospedeiros, um acesso exclusivo aos nutrientes de solo (Molina *et al.* 1992).

Por outro lado, as plantas variam consideravelmente na capacidade de se associarem a espécies de fungos (Richard *et al.* 2004). De uma maneira geral, as árvores que estabelecem associações com mais espécies estarão, em princípio, melhor adaptadas para subsistirem em condições adversas, uma vez que as diferenças interespecíficas se traduzem, muitas vezes, em diferenças funcionais (Cairney 1999; Luoma *et al.* 2004).

O reconhecimento da importância vital dos fungos ECM nos sistemas florestais impulsionou o desenvolvimento de vários estudos, em que se pretende a individualização e identificação das espécies de fungos ECM que irão constituir os potenciais inóculos de plântulas hospedeiras (e.g. Lu *et al.* 1999; Rincón *et al.* 1999), e assim contribuir para o sucesso das plantas micorrizadas.

Para além do papel vital dos fungos, como organismos, no equilíbrio dos ecossistemas, a maioria das espécies ECM e algumas sapróbias, incluídas principalmente na Divisão *Basidiomycota*, investem, durante a reprodução sexuada, em estruturas reprodutivas (Pilz & Molina 2001), de entre as quais se destacam os cogumelos comestíveis, por constituírem, actualmente, um recurso natural renovável e apresentarem um elevado interesse comercial (Pilz & Molina 2001; Yun & Hall 2004). Na realidade, para além do seu elevado valor gastronómico, constituem, em muitos países, uma componente fundamental da dieta alimentar, são utilizados com fins medicinais e representam um papel crucial em cerimónias e rituais (Yun & Hall 2004). Desta forma, a importância deste recurso varia geograficamente, de acordo com as tradições culturais de cada região (Pilz & Molina 2001).

Estas estruturas desenvolvem-se em condições climáticas favoráveis, segundo um padrão sazonal específico, de acordo com a fenologia das espécies. Os estudos de Bills *et al.* (1986), Brunner *et al.* (1992), Matsuda & Hijii (1998), Kranabetter & Kroeger (2001), Straatsma *et al.* (2001), Salerni *et al.* (2002), Giachini *et al.* (2004) sugerem que os factores abióticos, como a precipitação e temperatura, definem os padrões de ocorrência e abundância de carpóforos. O´Dell *et al.* (1999) acrescentou que a riqueza específica e a produtividade de carpóforos apresentam uma distribuição "unimodal" em função da precipitação média anual.

No entanto, apesar do padrão fenológico de cada espécie, assiste-se, geralmente, a uma variação anual da composição das espécies que frutificam e da produção de carpóforos num mesmo local (Bills *et al.* 1986; Dahlberg *et al.* 1997; O´Dell *et al.* 1999; Straatsma *et al.* 2001; Straatsma & Krisai-Greilhuber 2003; Bonet *et al.* 2004), o que significa que o investimento nestas estruturas está igualmente relacionado com factores intrínsecos à própria espécie.

De facto, o desenvolvimento de corpos de frutificação pelas espécies de fungos é difícil de prever, resultando, directamente, de uma conjugação e interacção de factores, que se revele favorável ao investimento e opção por uma reprodução sexuada.

A presença das espécies num determinado local e a consequente frutificação das mesmas dependem de inúmeros parâmetros ambientais, que se revelam mais ou menos importantes de acordo com as características próprias das espécies ou dos grupos tróficos a que pertencem.

No entanto, o processo de colonização varia, de uma maneira geral, com a composição, densidade dos estratos e/ou estado de maturação das espécies vegetais (Bills *et al.* 1986; Villeneuve *et al.* 1989; Brunner *et al.* 1992; Matsuda & Hijii 1998; Laganà *et al.* 1999; Lu *et al.* 1999; Kernaghan & Harper 2001; Smith *et al.* 2002; Jones *et al.* 2003; Moreau & Courtecuisse 2003; Bonet *et al.* 2004; Giachini *et al.* 2004; Richard *et al.* 2004; Richard *et al.* 2005), características edáficas (Villeneuve *et al.* 1989; Baar 1996; Lu *et al.* 1999; Kernaghan & Harper 2001; Lilleskov *et al.* 2001; Peter *et al.* 2001a; Avis *et al.* 2003; Trudell & Edmonds 2004) e características geomorfológicas do local (Laganà *et al.* 1999; Moreau & Courtecuisse 2003; Bonet *et al.* 2004), entre outros.

A presença das espécies de macrofungos pode ser facilmente inferida através da frutificação das mesmas, no entanto a inexistência de carpóforos num determinado ecossistema ou período sazonal, não indica, necessariamente, uma ausência, ao nível subterrâneo, da espécie produtora desse mesmo carpóforo.

Na realidade, alguns estudos que compararam a comunidade de fungos ECM produtora de carpóforos com a comunidade presente ao nível radicular observaram uma fraca correlação entre a diversidade e composição específica de fungos (Gardes & Bruns 1996; Dahlberg *et al.* 1997; Peter *et al.* 2001b; Richard *et al.* 2005). A relação entre a frequência e abundância de carpóforos e micorrizas varia com a espécie e poderá estar relacionada com padrões de alocação de recursos e/ou requisitos ecológicos específicos (Gardes & Bruns 1996; Walker & Miller 2002).

- FACTORES DE AMEAÇA

Dada a importância das características ambientais na colonização e frutificação dos fungos, a alteração dos habitats inerente ao desenvolvimento e, particularmente, à industrialização contribuiu, nos últimos anos, para um declínio acentuado da riqueza de fungos na Europa e, principalmente, dos fungos

ECM (Arnolds 1991; Courtecuisse 2001). O aumento da poluição atmosférica e a redução de habitats constituem os principais factores responsáveis por este declínio (Courtecuisse 2001).

Por outro lado, verificou-se que a implementação de novas medidas de gestão dos ecossistemas, que implicaram o corte das árvores, excessivas mobilizações do solo, selecção das espécies de árvores, alterações de gestão do sub-coberto, fogo, fertilização, uso de pesticidas e pastoreio, se traduziram numa degradação física e microbiológica dos solos, influenciando, por sua vez, a presença, reprodução e produtividade dos fungos (Courtecuisse 2001; Pilz & Molina 2001; Wiensczyk *et al.* 2002). Alguns estudos demonstraram que o nível de distúrbio ou interferência no sistema natural influencia a estrutura e dinâmica das comunidades de fungos (Guidot *et al.* 2002; Jones *et al.* 2003; Luoma *et al.* 2004).

Na realidade, a reacção das comunidades de fungos a perturbações externas introduzidas no ecossistema é tão rápida, que estes poderão ser considerados como bioindicadores da estabilidade (e.g. Arnolds 1991; Trudell & Edmonds 2004).

Adicionalmente, a valorização económica dos cogumelos no mercado mundial aliada a uma incapacidade generalizada de produzir as espécies ECM em laboratório (*ex-situ*), contribui para um aumento da pressão da colheita de cogumelos, realizada, muitas vezes, de forma desregrada e abusiva (Pilz & Molina 2001) e consequentemente para a diminuição das espécies nos ecossistemas naturais.

- LISTA VERMELHA E PLANOS DE CONSERVAÇÃO

Desta forma, face a este declínio e a um aumento do interesse pela biodiversidade de fungos nos últimos anos, procedeu-se, em alguns países, à compilação de listas com espécies raras ou ameaçadas (Dahlberg & Croneborg 2003). Actualmente existem cerca de 35 países europeus que desenvolveram uma "Lista Vermelha" (Red List) provisória para os fungos. Em 2001, 33 espécies de fungos foram propostas para a inclusão na Convenção de Berna, no Anexo II, de forma a ser reconhecida a necessidade de conservar os fungos e os seus habitats. Todos os fungos propostos são raros na Europa e pertencem à "Lista Vermelha" de vários países (Dahlberg & Croneborg 2003). Em Portugal, existem 10 espécies incluídas na lista, as quais foram detectadas em 35% dos habitats Natura 2000. Contudo, não existe ainda nenhuma "Lista Vermelha" proposta para o nosso país, dada a escassez de estudos científicos realizados neste domínio.

A necessidade de conservar os fungos é tão inadiável, que estão a ser desenvolvidos esforços no sentido de inferir a comunidade de macrofungos, em determinado ecossistema, através das espécies arbóreas presentes, uma vez que a protecção das áreas seleccionadas pelas espécies arbóreas deverá resultar na protecção das espécies de macrofungos (Schmit *et al.* 2005).

A avaliação do estatuto de ameaça de cada espécie de macrofungos dependerá, numa primeira fase, da realização de inventários em diversos ecossistema, antrópicos ou naturais, que irão contribuir para o enriquecimento do conhecimento da biologia, ecologia e distribuição dessa mesma espécie (Courtecuisse 2001; Pilz & Molina 2001; Wiensczyk *et al.* 2002).

De acordo com Courtecuisse (2001), será irreal desenvolver planos direccionados exclusivamente para a protecção individual de fungos, como acontece para as plantas ou animais. As estratégicas a implementar poderão incidir numa conservação *in situ*, através da protecção dos habitats naturais, delimitação de reservas micológicas, delimitação de corredores ecológicos ou conservação *ex-situ*, mais eficaz para as espécies sapróbias.

Por outro lado, os planos de gestão que valorizarem a protecção das comunidades de macrofungos, de uma forma directa ou indirecta, estão a contribuiu para o aumento da biodiversidade da micobiota e, consequentemente, estão a favorecer a estabilidade de todo o ecossistema e a maximização dos recursos naturais.

- Situação em Portugal

Apesar da importância ecológica e económica dos fungos, predomina, em Portugal, um desconhecimento generalizado acerca do património etnomicológico e da diversidade específica de fungos, particularmente das espécies que colonizam os diversos habitats, das espécies ameaçadas que urge proteger e das espécies potenciais que poderão constituir um recurso natural economicamente rentável.

Este desconhecimento potencia a gestão desadequada dos ecossistemas, com vista a uma maior rentabilização dos sistemas, que, por vezes, se traduz em situações de declínio. Esta situação é ainda mais crítica no Sul do País.

SISTEMA DE MONTADO

- FACTORES DE AMEAÇA

No Sul de Portugal e, especificamente no Alentejo, o montado de sobro (*Quercus suber* L.), dominante na paisagem, representa um exemplo de um sistema humanizado associado a práticas de cultivo extensivo ecologicamente sustentáveis (Scarascia-Mugnozza *et al.* 2000; Azul, 2002; Díaz *et al.*, 2003), que revela sintomas de declínio, seja por situações de abandono e/ou substituição por outras culturas, seja por intensificação do sistema (descortiçamento precoce, uso intensivo do solo) (Azul 2002). De facto, não obstante ao seu valor económico e ecológico, a redução das áreas de montado imposta pela implementação de estratégias economicistas de exploração e de produção intensivas, traduziu-se numa simplificação da paisagem, que, por sua vez, conduziu à redução de reservas de água, à destruição de *habitats*, ao esgotamento dos solos, à perda de biodiversidade, à erosão e à desertificação (Azul 2002).

A manutenção do sobreiro, como componente integrante do coberto vegetal no Alentejo, proporciona vantagens sob o ponto de vista económico, sobretudo através da exploração da cortiça, e ecológico, na medida em que previne a degradação do solo, muito susceptível a fenómenos erosivos (Azul 2002).

- IMPORTÂNCIA DOS FUNGOS NA CONSERVAÇÃO DOS SISTEMAS COM SOBREIRO

Desta forma, a maximização da rentabilização económica dos sistemas dominados por sobreiro dependerá, certamente, de uma exploração sustentada, que integre medidas de protecção e de conservação da comunidade de fungos, optimizando a produção de carpóforos e estabilização das comunidades ao nível radicular e consequentemente o equilíbrio do sistema. Esta dupla importância de alguns fungos reflecte-se, de uma forma directa ou indirecta, na economia local.

Neste contexto, o presente estudo é mais um contributo para a inventariação micológica da região do Alentejo, incidindo, especificamente, num sistema representativo da paisagem dominante da região – montado de sobro (*Quercus suber*) - e avaliação das condições microambientais que maximizam a potencialidade micológica, no Sítio de Monfurado.

Com efeito, apesar do sistema ser dominado pela mesma espécie hospedeira, é sujeito, frequentemente, a diversas práticas florestais, que interferem nas características ambientais do sistema, nomeadamente na penetração de luz e vento, temperatura ambiente e do solo, conteúdo hídrico e orgânico do solo, compactação do solo, entre outras.

Assim, assumindo o pressuposto que as condições ecológicas do habitat afectam, principalmente, a presença e frutificação de espécies de fungos, pretende-se, especificamente, com este estudo, avaliar as condições ambientais que optimizem a presença, dinâmica e frutificação destas mesmas comunidades, propondo estratégias de gestão e conservação.

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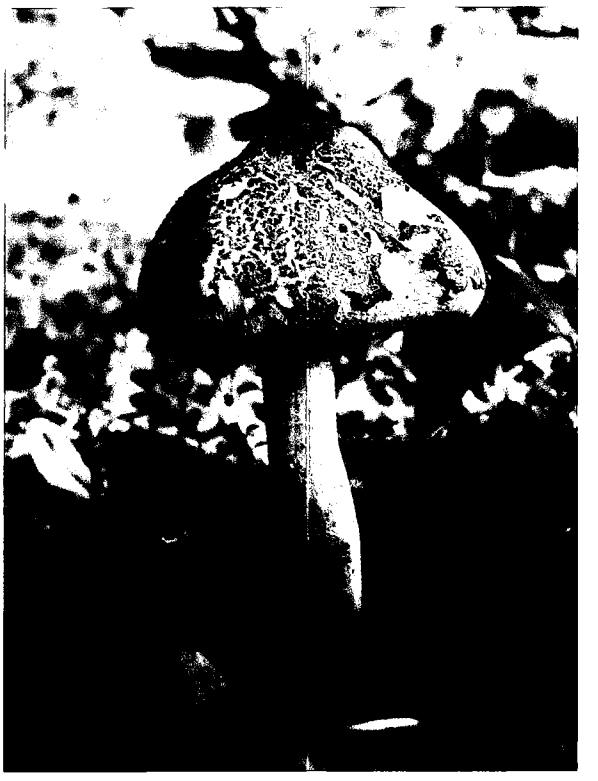
II. ARTIGO CIENTÍFICO



SPECIES RICHNESS AND PRODUCTIVITY OF EPIGEOUS FUNGAL SPOROCARPS IN A CORK OAK (*QUERCUS SUBER* L.) STAND IN SOUTHERN PORTUGAL – RELATION WITH MICRO-ENVIRONMENTAL FACTORS

ABSTRACT

The present study aimed to contribute to the characterization of macro-fungal communities in a cork oak (*Quercus suber* L.) *montado* (open forest) ecosystem and to evaluate micro-environmental conditions that favour occurrence and fructification of those communities. With this purpose, four sampling plots were selected, with the same exclusive host tree (*Quercus suber*) and similar climatic conditions but subject to different management strategies, in terms of system stratification and livestock pressure. Methodology focused exclusively on sporocarp sampling, during one fructification period (2005-2006).

Results revealed a preferential fruiting period in late Autumn and a significantly positive correlation ($r = 0.89$; $p < 0.01$) between species richness and total productivity values during the study period. In the *montado* ecosystem 59 species were identified, included in 30 genera, with a higher representation of saprobic species (76 %). Landscape simplification, imposed by reduced tree cover, absence or reduced representation of shrub under-storey and increased soil compaction and nitrogen content inherent to active livestock presence, result in particular ecological and micro-environmental conditions that could be more limitary to macro-fungal communities. However, analysis and comparison between sampling plots showed distinct total species richness and proportions of trophic groups, a low percentage of shared species (8 %), a considerable proportion of exclusive species (52 %). Data obtained suggest that particular micro-environmental conditions influence colonization and fructification of macro-fungal communities and, specifically, that ectomycorrhizal (ECM) fungus are favoured more in areas with more vegetation complexity, a higher number of host trees and lower nitrogen contents, while saprobic fungi appear preferentially in more open areas and nitrogen-rich substrata.

Sustainable ecosystem management, with regard to community equilibrium and higher economic valuation, should focus more on favouring ECM species, assuring host protection and health, maximizing the quality of its sub-products (cork and acorn) and the probable fructification of edible sporocarps with high economic and gastronomic value. Several management practices will be suggested.

KEY WORDS

Macrofungi, mediterranean ecosystem, management, fruiting patterns, sustainability

The need to protect and preserve biodiversity and natural resources is becoming a major concern and priority among the scientific community and, increasingly, in general society, in response to constant threats and negative impacts inherent to industrial development. Protection of biological communities and ecosystem functional maintenance will be more effective with a holistic approach, integrating biological, ecological, geophysical, silvicultural and socio-economic information while meeting human needs (Pilz & Molina 2001). In this sense, any management plan depends directly on previous scientific studies and, particularly, on inventories and dynamic evaluation of populations in several ecosystems, with the purpose of strategic optimization (Courtecuisse 2001).

The focus of these studies on mycobiotic communities and especially on macro-fungi becomes crucial, because they assume a fundamental role in ecosystem balance, contributing to the turnover of organic matter, favouring establishment of plant species in adverse conditions through symbiotic associations or, inclusively, by eliminating less healthy species (e.g. Alexopoulos & Mims 1979; Smith & Read 1997; Mueller & Bills 2004). Moreover, fungal communities may act as bio-indicators of ecosystem stability, since they respond rapidly to negative impacts (e.g. Arnolds 1991; Trudell & Edmonds 2004). In addition, some species invest in reproductive structures – mushrooms – that are considered gastronomic delicacies, highly valued worldwide (Pilz & Molina 2001; Yun & Hall 2004).

The presence of species in a particular ecosystem and consequent investment in the fruiting process depend on several environmental parameters, which can be more or less important according to the intrinsic characteristics of each species or trophic group. Colonization processes vary, generally, with plant species composition, age and layer density (Bills *et al.* 1986; Villeneuve *et al.* 1989; Brunner *et al.* 1992; Matsuda & Hijii 1998; Laganà *et al.* 1999; Lu *et al.* 1999; Kernaghan & Harper 2001; Smith *et al.* 2002; Jones *et al.* 2003; Moreau & Courtecuisse 2003; Bonet *et al.* 2004; Giachini *et al.* 2004; Richard *et al.* 2004; Richard *et al.* 2005). Soil characteristics (Villeneuve *et al.* 1989; Baar 1996; Lu *et al.* 1999; Kernaghan & Harper 2001; Lilleskov *et al.* 2001; Peter *et al.* 2001a; Avis *et al.* 2003; Trudell & Edmonds 2004) and geomorphology (Laganà *et al.* 1999; Moreau & Courtecuisse 2003; Bonet *et al.* 2004) are just as influential.

Considering the importance of environmental characteristics for macro-fungal communities, the increase in air pollution or habitat fragmentation resulting from industrial development contributed, in recent years, to a significant decrease in the richness of fungal species in Europe, particularly of ectomycorrhizal

(ECM) fungi. The actual impact of these negative factors is still not quite well understood, since total species diversity of fungi is still unknown, although it was estimated in 1.5 millions species (Hawksworth 1991; Hawksworth 2001).

The scarcity of scientific studies carried out on this subject in the Mediterranean region, in Portugal in particular, reveals a relatively widespread lack of knowledge of ethno-mycological heritage and species richness, predominantly of those species that colonize diverse habitats, of threatened species and of edible species potentially exploitable as a natural resource.

This ignorance facilitates human intervention in ecosystems aimed at maximizing economic value, which often turns out to be prejudicial to macro-fungal communities. In fact, logging methods and clearcut harvesting, excessive soil mobilization, tree species selection, changes in understorey management, fire, fertilization, pesticide use and grazing may lead to a physical and microbiological degradation of soils, with influence on the presence, reproduction and productivity of fungi (Courtecuisse 2001; Pilz & Molina 2001; Wiensczyk *et al.* 2002). The predominance and/or disappearance of certain species will depend on their spreading ability and host specificity (Azul 2002).

The open forest of cork oak, *Quercus suber* L., designated as cork oak *montado,* which dominates the landscape of southern Portugal, illustrates a humanized system related to extensive and ecologically sustainable agricultural practices that have been decreasing in recent years, because of abandonment, substitution or intensification of those practices (Azul 2002). These semi-artificial systems result from the clearing of Mediterranean woods and the maintenance of grazing and understorey agricultural practices (Azul 2002). Reversion of this tendency will depend on the implementation of sustainable land use plans that consider existing macro-fungal communities.

Evaluation of colonizing macro-fungal communities in any ecosystem may be carried out by analyzing vegetative or reproductive structures of fungi. Sporocarp sampling is the most commonly applied method, despite some limitations: 1) it requires long-term monitoring (Perini *et al.* 1996; O'Dell *et al.* 1999; Schmit *et al.* 1999; Straatsma *et al.* 2001; Straatsma & Krisai-Greilhuber 2003; Bonet *et al.* 2004), given the influence of environmental parameters and intrinsic, specific characteristics of sexual reproduction; 2) it does not allow the detection of ECM species that do not invest in sporocarps (such as *Cenococcum geophilum*) or that develop inconspicuous ones, reflecting only a part of the ECM community composition of root systems (Gardes & Bruns 1996; Peter *et al.* 2001b; Richard *et al.* 2005); 3) there is a notable absence of method standardization or optimization (Schmit *et al.* 1999).

Even so, according to some authors, sporocarp sampling represents an advantageous and feasible method for characterizing macro-fungal communities (e.g. Dahlberg *et al.* 1997; Straatsma *et al.* 2001; Straatsma & Krisai-Greilhuber 2003; Richard *et al.* 2004).

Therefore, the present study focussed on the evaluation of a macro-fungal community in a cork oak *montado* ecosystem, using sporocarp sampling, with two main purposes:

- To contribute to mycological inventories of southern Portugal (Alentejo region);
- To determine micro-environmental parameters that optimize and maximize the mycological potential of this ecosystem, through comparison of species richness and sporocarp productivity in areas with one host-tree species and subject to similar climatic conditions, soil, aspect and altitude but with different micro-environmental characteristics, thereby proposing strategies for management and conservation.

MATERIAL AND METHODS

Study area

The present study was performed at Herdade de João Pais, Sítio Monfurado, located in central Alentejo, Southern Portugal (Figure 1), involving areas from the municipalities of Montemor-o-Novo and Évora.

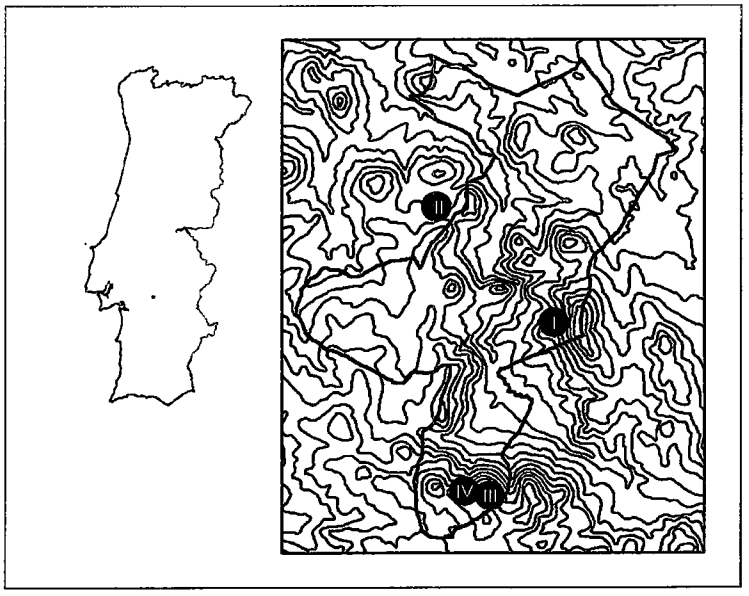


Figure 1. Study area.

The altitude varies between 150 m and 420 m and the landscape is dominated by important cork and round-leafed (holm) oak *montado* ecosystems, well preserved, with predominant grazing by bovine

livestock. Sítio Monfurado has a Mediterranean climate characterized by rainy cool winter and long hot summer seasons, with at least two dry months (Pereira 2002).

According to the 1951-1980 climatological standard registered at the nearest station (Évora-Mitra) the mean annual temperature was 15.4 ºC and rainfall 665 mm; the coldest and rainiest month was January (mean minimum temperature 3.8 ºC and maximum rainfall 98 mm) and the warmest and driest one was August (maximum mean temperature 31.3 ºC and minimum rainfall 3 mm) (Mendes *et al.* 1991).

Sampling design

Four permanent sampling plots were selected (Figure 1), each with an area of 1200 m^2 (30 m x 40 m), subject to different land use: I – dispersed distribution of host trees, with some shrubs; II - dispersed distribution of host trees, without shrubs; III – denser distribution of host trees and shrubs; IV – dense distribution of host trees with less shrubs.

Environmental characterization of each plot was carried out: specifically, stand structure (number of trees, maximum height, DBH and canopy radius), percentage of cover of different vegetation layers and shrub species present. Additionally, grazing presence and soil litter thickness were quantified. Soil samples (0 cm - 10 cm depth, 10 replicas) were collected from each plot in order to determine total organic matter, nutrient content and pH.

Sporocarp survey

A sporocarp survey was conducted during the fruiting season from October 2005 to April 2006, at regular intervals of one or two weeks, depending on fruiting intensity, since the intention was not to sample the entire mycological community but rather to make a comparison of sporocarp-producing species richness and productivity between the four sampling plots.

In each sampling survey all epigeous fungi species sporocarps (except *Ascomycota* and *Gasteromycetes*) in good condition were identified to the lowest taxonomic level possible, counted and mapped. Specimens for which identification was doubtful in the field were collected and identified in the laboratory, using diverse available literature (e.g. Moser 1983; Moreno *et al.* 1986; Breitenbach & Kränzlin 1991, 1995, 2000; Courtecuisse & Duhem 1995; Frade & Alfonso 2003; Kränzlin 2005). All recent species

names were confirmed in *Index Fungorum*, since some incongruence in synonymy still exists (Kirk & Cooper, 2007).

Only the sporocarps strictly necessary were removed, in order to minimize negative impacts on the sampling sites.

Sporocarps of saprobic or parasitic macro-fungi fruiting directly on woody debris, excrements or living plants were counted, despite their dependence on the occasional occurrence of such substrata.

In addition, a representative voucher collection was created and deposited in the herbarium, consisting of one specimen of each species found in the sampling area.

Identified *taxa* were grouped into three categories – edible, inedible or toxic, or without gastronomic value, according to the criteria of Frade & Alfonso (2003) whenever possible, since they follow a more Mediterranean evaluation.

Soil analysis

Soil analysis was performed by Laboratório Químico Agrícola, University of Évora.

For each sampling plot, pH, percentage of total organic matter and phosphorus (P_2O_5), nitrogen (NO_3) and potassium (K_2O) contents were measured.

Soil acidity (pH) was determined with a potentiometric method, in a 1:2.5 soil/water suspension (Piper 1950; Black *et al.* 1965).Measurements were performed in a CRISON, micro-pH 2001 potentiometer, with combined electrode, calibrated with pH 7 and pH 4 buffer solution.

Assimilable phosphorus and potassium were calculated using the Egner-Riehm method with an 1 N ammonium lactate and 0.4 N acetic acid buffered solution, with pH ranging between 3.65 and 3.75 (Riehm 1958). Extracts were prepared in a 1:20 soil solution, shaken for two hours at constant velocity and filtered. The resulting solution was prepared as follows:

- Phosphorus (P_2O_5), using molybdate antimony and potassium-ascorbic acid colorimetric analysis (Knudsen 1980), the measurements being taken with a UV/V HITACHI U-2000 spectrophotometer with a 650 nm wavelength;
- Potassium (K_2O), by means of flame photometry, using a JENWAY, PFP7 flame photometer.

Total nitrogen content was determined using the Kjeldahl Method (Bremner & Mulvaney 1982). Soil was digested (GERHARDT, Kjeldatherm) with concentrated sulphuric acid in the presence of a catalyst, transforming organic nitrogen into ammonium, which attaches to the acid directly linked to ammonium

sulphate. The digested solution was vapour distilled (GERHARDT, Vapodest 2) in a highly alkaline medium (NaOH 10 N), the released ammonium being collected in 4 % boric acid and titulated with H_2SO_4 0.1 N.

Total organic matter was determined using the Anne Method (Anne 1945), which consists of soil oxidation with 8 % potassium dichromate solution in an acid medium, at high temperature. Oxidant in excess was volumetrically dosed with an ammonium ferric sulphate solution of known titre.

Statistical analysis

To highlight the phenological pattern of the *montado* ecosystem's macro-fungal community, all sampling plots data (I, II, III and IV) were integrated and, for each sampling period, species richness (*S*) and total productivity were determined. Species richness corresponds to the total number of identified *taxa* and total productivity related to total number of sporocarps.

This procedure was applied, in the same way, to determine the phenological pattern of the macro-fungal community in each sampling plot.

The relation between fruiting patterns of the *montado* macro-fungal community and fruiting patterns of each sampling plot was evaluated using the Spearman correlation rank, given the non-normality of data. Moreover, species richness observed in each sampling period, on each sampling plot, was compared using a Friedman test, followed by *a posteriori* Simultaneous Tests Procedures (STP), according to Siegel & Castellan (1988).

Species composition of the *montado* ecosystem was obtained by integrating the data from each sampling plot. Frequency of sporocarp presence (%) and maximum productivity during the entire study period were determined for each species, in each sampling plot and in the whole *montado* ecosystem.

Similarity between sampling plots was assessed by the Jaccard index (*J*) (Pielou 1984), calculated as:

$$J = \frac{c}{a + b - c}, \qquad (1)$$

where *a* is the total number of species in the first community, *b* is the total number of species in the second community and *c* is the number of species present in both communities.

The Shannon-Wiener diversity index (*H'*) was also determined for each sampling period, for the four sampling plots. This index is based on species richness and on the number of specimens of each *taxon* (Legendre & Legendre 1979) according to the formula:

$$H' = -\sum_{i=1}^{S} pi \ln pi, \quad (2)$$

where S is the total number of taxa and, in this particular case, pi the proportion of sporocarps of taxon i relative to the total number of sporocarps.

To evaluate *taxa* spatial patterns a Principal Components Analysis (PCA) was performed, considering the maximum observed abundance distribution of each genus in the sampling plots and its relation with environmental variables. Correlated environmental variables were excluded from the analysis.

The statistical software package SPSS 14.0 was used to calculate descriptive statistics and perform statistical tests.

RESULTS

I. Environmental characterization of sampling plots

Edaphic and ecological parameters

The analysis of some ecological and edaphic parameters emphasized micro-environmental differences between sampling plots (Table 1).

Table1. Ecological and edaphic characterization of sampling plots

	Sampling Plots			
	I	II	III	IV
Altitude (m)	280	250	350	350
Aspect	0°	0°	0°	0°
Soil type	Less unsaturated alluviated clays	Less unsaturated alluviated clays	Less unsaturated alluviated clays	Less unsaturated alluviated clays
Livestock presence (%)	70	70	25	25
Tree number	8	7	21	32
Tree cover (%)	21	15	40	80
Shrub cover (%)	0.84	0	26	6
Herbaceous cover (%)	85	75	85	90
Tree medium height (m)	6.5	8	7.7	8.9
Canopy medium radius (m)	3.5	4	3.5	3.1
BHD (Breast height diameter)	37.5	35.3	26.9	29.2
Tree regeneration (no.)	10	0	101	193
Organic layer thickness (cm)	0.25	0.25	1	3
Phosphorus (P_2O_5) (PPM)	80	22	12	18
Nitrogen (NO_3) (PPM)	382	91	77	71
Potassium (K_2O) (PPM)	920	168	224	376
Organic matter (%)	4.5	4.7	9.2	7.3
C: N	18.8	9.4	57.5	11.8
pH (H_2O)	5.69	6.23	5.5	5.86

Climate parameters

During almost the entire study period a parallel was observed between variation of mean air temperature and total rainfall (Figure 2).

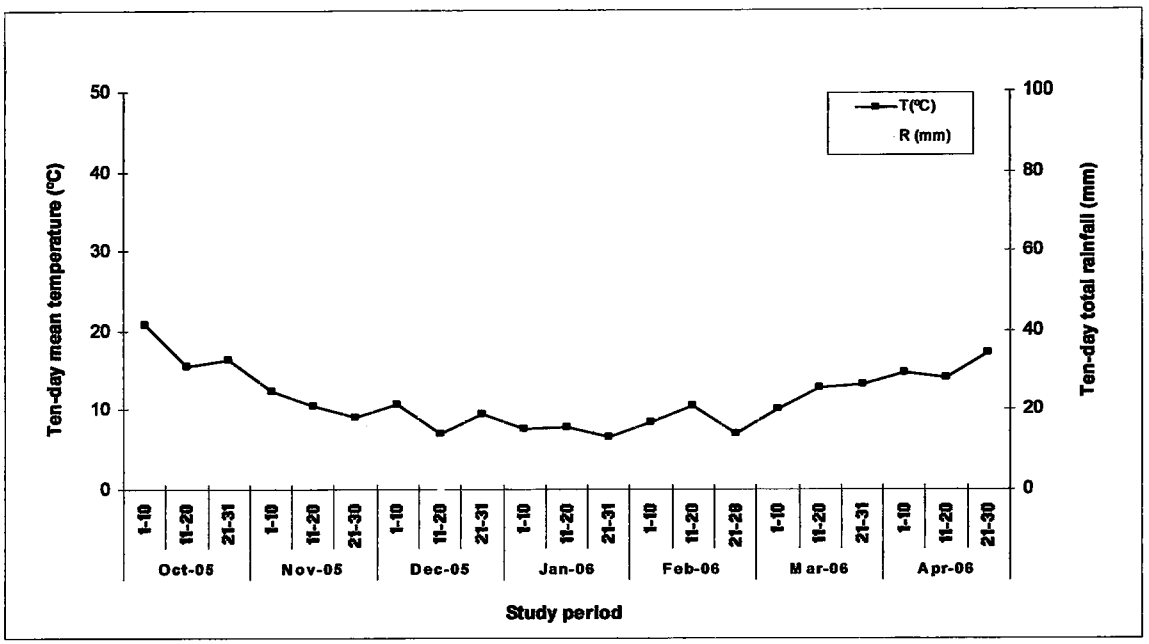


Figure 2. Ten-day mean temperature and total rainfall variation during the study period.

In general, temperature and rainfall gradually decreased until December 11 to 20, when the lowest values where measured. Nonetheless, during this period, significant fluctuations in both physical parameters occurred, with maximum peaks followed by abrupt falls. The time period until a new minimum value was reached – February 20 to 28 – showed less significant parameter variations, with emphasis on two maximum temperature and rainfall peaks (December 21 to 31; February 11 to 20). From March onwards, temperatures gradually increased, followed by rainfall until March 20. After this period, rainfall decreased drastically at first, then progressively.

II. General characterization of the macro-fungal community

Species richness and productivity during the study period

During the study period, 59 different *taxa*, included in 30 genera, were identified. However, the number of fruiting species and the total production of sporocarps in each sampling survey varied with time, reflecting the species phenology.

Graphical analysis showed an obvious parallel between the variation of species richness and total productivity with time (Figure 3).

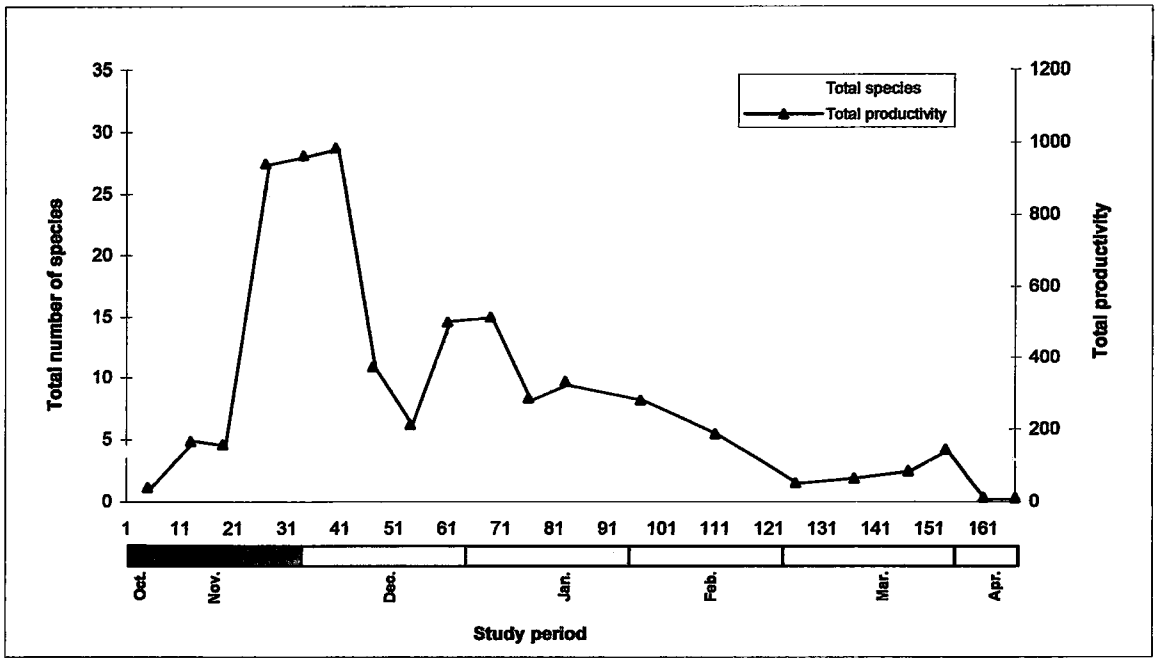


Figure 3. Total species richness and productivity variation during the study period.

Sequentially, both parameters reached maximum peaks (November 16 to December 6, 2005) followed by a sudden fruiting decrease (December 7 to 20, 2005), then a second, but less intense, peak (December 21, 2005 to January 4, 2006), a gradual diminution (January 5 to March 23, 2006), a last, almost imperceptible, peak (March 24 to 30, 2006) and again a decline until the end of the study period.

At the maximum fruiting peak, 33 species were identified and 986 sporocarps counted.

The Spearman correlation rank between total productivity and species richness in each sampling survey is significant ($r = 0.90$; $p < 0.01$).

Taxa representativeness – presence, productivity and edibility

An integrated analysis of data obtained from the four sampling plots showed that the majority of identified species were *Agaricaceae* (24 %) or *Tricholomataceae* (22 %) (Table 2).

Table 2. Family representativeness (%) in the *montado* ecosystem

	FAMILY REPRESENTATIVENESS (%)
Agaricaceae	24
Tricholomataceae	22
Bolbitiaceae	12
Cortinariaceae	10
Russulaceae	8
Strophariaceae	7
Psathyrellaceae	5
Pluteaceae	3
Marasmiaceae	3
Hydnangiaceae	2
Entolomataceae	2
Boletaceae	2

Species with the most extensive fruiting period and, consequently, with higher percentages of sporocarp occurrence during the study period (> 50 %) were *Coprinus comatus* (100 %), *Panaeolus campanulatus* (81 %), *Panaeolus acuminatus* (71 %), *Melanoleuca exscissa* (71 %), *Clitocybe obsoleta* (67 %), *Psathyrella hirta* (62 %), *Laccaria laccata* (57 %), *Entoloma sericeum* (57 %), *Lepista flaccida* (52 %) and *Agaricus* sp. 1 (52 %).

However, species with the highest sporocarp production (> 50 sporocarps), at the maximum peak of the fruiting period, considering all sampling plots, were *Coprinus comatus* (65), *Lepista flaccida* (68), *Panaeolus acuminatus* (84), *Panaeolus campanulatus* (89), *Laccaria laccata* (269) and *Clitocybe obsoleta* (618).

Concerning sporocarp edibility, species with no commercial interest as a natural resource were dominant (72 %), either because they were toxic or presented unpleasant organoleptic properties or their biomass and/or taste were irrelevant (Tabela 3)

Table 3. Classification and representativeness of identified species according to edibility

	REPRESENTATIVENESS (%)
Edible	28
Inedible or toxic	52
Without gastronomic value	20

Among edible species, two groups were distinguished – good and mediocre - in which the first represented 12 % and the second, 16 % of the species.

Coprinus comatus is commonly accepted as an excellent edible mushroom and was observed in all sampling plots, fruiting during the entire study period and with a considerable sporocarp production.

III. Characterization and comparison of macro-fungal community in each sampling plot

Species composition

Each of the four sampling plots revealed a characteristic list of species, despite some similarities (Table 4).

Table 4. List of identified *taxa* in each sampling plot: frequency of sporocarp occurrence during the study period and maximum productivity peak of each *taxon*

IDENTIFIED *TAXA*	SAMPLING PLOTS							
	I		II		III		IV	
	Presence frequency (%)	Maximum productivity	Presence frequency (%)	Maximum productivity	Presence frequency (%)	Maximum productivity	Presence frequency (%)	Maximum productivity
Agaricus bisporus (J.E. Lange) Pilát	5	1	0	0	0	0	0	0
Agaricus campestris var. *campestris* L.	10	3	0	0	0	0	0	0
Agaricus essettei Bon	0	0	0	0	5	1	0	0
Agaricus moelleri Wasser	0	0	0	0	0	0	5	2
Agaricus sp. 1	0	0	52	13	0	0	0	0
Agaricus sp. 2	0	0	0	0	0	0	29	13
Agaricus sp. 3	0	0	0	0	0	0	5	2
Amanita vaginata (Bull.) Lam.	0	0	0	0	5	1	0	0
Armillaria mellea (Vahl) P. Kumm.	10	26	0	0	14	2	19	5
Bolbitius vitellinus (Pers.) Fr.	5	2	0	0	0	0	0	0
Bolbitius vitellinus var. variicolor (G.F. Atk.) Krieglst.	14	4	0	0	0	0	0	0
Boletus chrysenteron Bull.	0	0	0	0	0	0	5	1
Clitocybe costata Kühner & Romagn.	5	1	5	5	19	10	0	0
Clitocybe gibba (Pers.) P. Kumm.	5	1	14	7	29	22	5	1
Clitocybe obsoleta (Batsch) Quél.	62	207	67	495	5	4	5	1
Clitocybe subspadicea (J.E. Lange) Bon & Chevassut	5	1	0	0	0	0	0	0
Conocybe albipes Hauskn.	19	4	0	0	0	0	0	0
Coprinus auricomus Pat.	5	3	0	0	0	0	0	0
Coprinus comatus (O.F. Müll.) Gray	90	29	81	22	5	1	10	1
Coprinus picaceus (Bull.) Gray	0	0	0	0	0	0	10	1
Coprinus plicatilis (Curtis) Fr.	14	15	10	4	0	0	0	0
Coprinus xanthothrix Romagn.	0	0	0	0	0	0	5	1
Cortinarius decipiens var. decipiens (Pers.) Fr.	0	0	5	2	0	0	19	3
Entoloma sericeum (Bull.) Quél.	14	4	33	12	33	4	24	4
Gymnopilus suberis (Maire) Singer	0	0	0	0	0	0	5	4
Gymnopus erythropus (Pers.) Antonín, Halling & Noordel.	0	0	0	0	10	9	0	0
Inocybe bresadolae Massee	0	0	0	0	5	4	5	1
Inocybe geophylla var. geophylla (Pers.) P. Kumm	0	0	5	2	0	0	5	1

(continued)

Inocybe geophylla var. lilacina Gillet	0	0	10	1	0	0	0	0
Laccaria laccata (Scop.) Fr.	10	3	19	7	38	72	52	249
Lactarius camphoratus (Bull.) Fr.	0	0	0	0	5	1	5	1
Lactarius lacunarum Romagn. ex Hora	0	0	0	0	0	0	14	3
Lactarius rugatus Kühner & Romagn.	0	0	0	0	0	0	10	2
Lepista flaccida (Sowerby) Pat.	0	0	0	0	52	68	0	0
Lepista nuda (Bull.) Cooke	19	11	0	0	14	2	5	1
Lepista sordida (Fr.) Singer	5	1	24	29	0	0	0	0
Macrolepiota fuligineosquarrosa Malençon	0	0	5	1	0	0	0	0
Macrolepiota konradii (Huijsman ex P.D. Orton) M.M. Moser	5	1	0	0	29	8	14	1
Melanoleuca exscissa var. exscissa (Fr.) Singer	71	48	0	0	0	0	0	0
Mycena haematopus (Pers.) P. Kumm.	14	4	0	0	0	0	10	2
Mycena pura (Pers.) P. Kumm.	0	0	0	0	5	5	0	0
Naucoria bohemica Velen.	10	2	0	0	0	0	0	0
Omphalina pyxidata (Bull.) Quél.	0	0	43	12	10	14	0	0
Panaeolus acuminatus (Schaeff.) Quél.	57	70	52	12	0	0	33	4
Panaeolus campanulatus (L.) Quél.	62	52	52	36	0	0	24	2
Panaeolus fimicola (Pers.) Gillet	19	5	0	0	5	3	0	0
Psathyrella candolleana (Fr.) Maire	0	0	0	0	5	1	24	3
Psathyrella hirta Peck	24	30	48	25	0	0	0	0
Psathyrella spadiceogrisea (Schaeff.) Maire	0	0	0	0	5	1	14	2
Pseudoclitocybe cyathiformis (Bull.) Singer	10	13	0	0	0	0	0	0
Psilocybe coprophila (Bull.) P. Kumm.	14	24	24	18	0	0	5	2
Psilocybe crobula (Fr.) Singer	0	0	0	0	10	1	0	0
Rhodocollybia butyracea f. butyracea (Bull.) Lennox	0	0	0	0	19	12	24	3
Russula amoena Quél.	0	0	0	0	5	1	0	0
Russula pectinatoides Peck	0	0	14	21	10	3	19	23
Stropharia luteonitens (Vahl) Quél.	5	1	0	0	0	0	0	0
Stropharia semiglobata (Batsch) Quél.	5	1	29	2	0	0	0	0
Tubaria hiemalis Romagn. ex Bon	5	6	29	30	0	0	5	1
Volvariella gloiocephala (DC.) Boekhout & Enderle	19	3	0	0	0	0	0	0
Total Species	**31**		**21**		**24**		**30**	

Species richness and taxonomic representation

Considering the complete study period, a total richness was observed of 31 species (20 genera), 21 species (19 genera), 24 species (19 genera) and 30 species (21 genera) in sampling plots I, II, III and IV, respectively. *Agaricus* sp. 1, 2 and 3 were included in the species list because the identification process allowed the conclusion that they were different species, incorporated in distinct groups.

The species most often observed in sampling plots I, II and III belong to *Tricholomataceae*, with 9 (30 %), 5 (23 %) and 8 (34 %) species, respectively; in sampling plot IV *Agaricaceae* are predominant, with 7 (24 %) species (Figure 4).

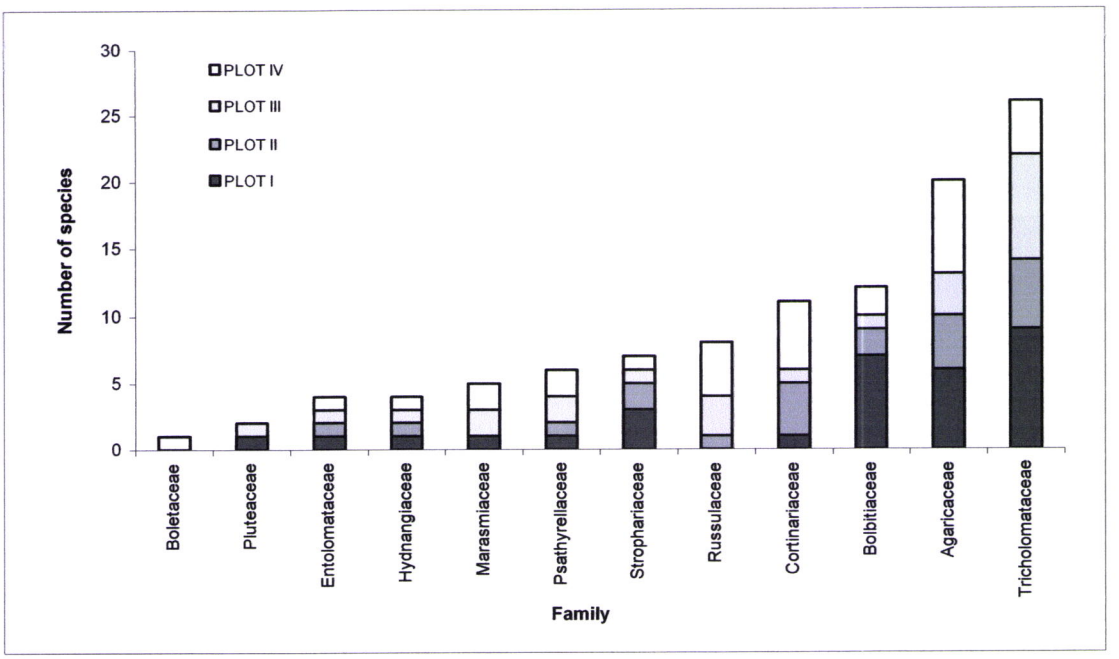


Figure 4. Number of species included in each Family, in each sampling plot.

Exclusive and shared species between sampling plots

Of a total of 59 species identified, 20 % (12 species), 5 % (13 species), 12 % (7 species) and 15 % (9 species) are exclusive to sampling plots I, II, III and IV, respectively.

The most common and widespread species, found in every sampling plot, at least once during the study period, represent 8 % of the total number of species and include *Clitocybe gibba*, *Clitocybe obsoleta*, *Coprinus comatus*, *Entoloma sericeum* and *Laccaria laccata*.

The Jaccard index revealed the highest similarities between sampling plots I and II (J = 0.37) and between III and IV (J = 0.35), regarding shared species.

Maximum productivity

Species that produced more sporocarps (> 50) in a specific time period differ among sampling plots: *Clitocybe obsoleta* (207), *Panaeolus acuminatus* (70) and *Panaeolus campanulatus* (52) in plot I; *Clitocybe obsoleta* (495) in plot II; *Laccaria laccata* (72) and *Lepista flaccida* (68) in plot III; *Laccaria laccata* (249) in plot IV (Table 4).

Diversity

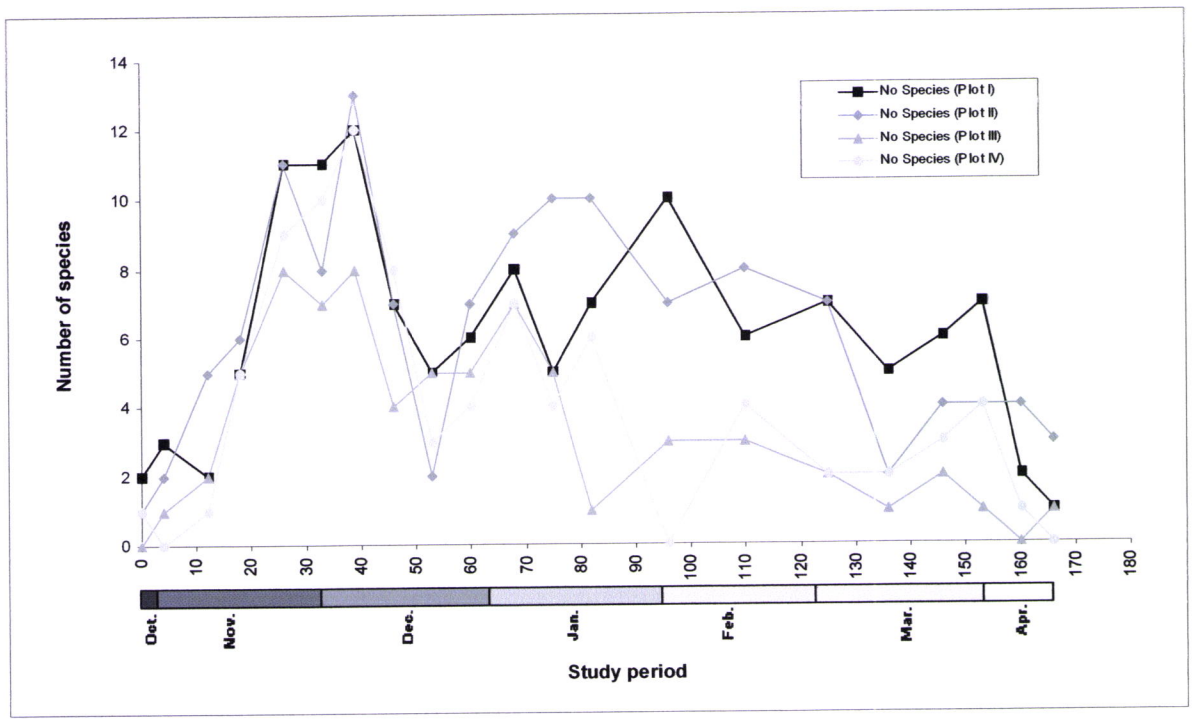
The Shannon-Wiener diversity index (*H'*) fluctuated considerably throughout the study period, reaching distinct maximum peaks in the different sampling plots. Nevertheless, comparison of maximum peaks of each sampling plot showed highest diversity in plot I (1.79) and lowest diversity in plot III (1.37).

Species with highest frequency of occurrence/longest fruiting period

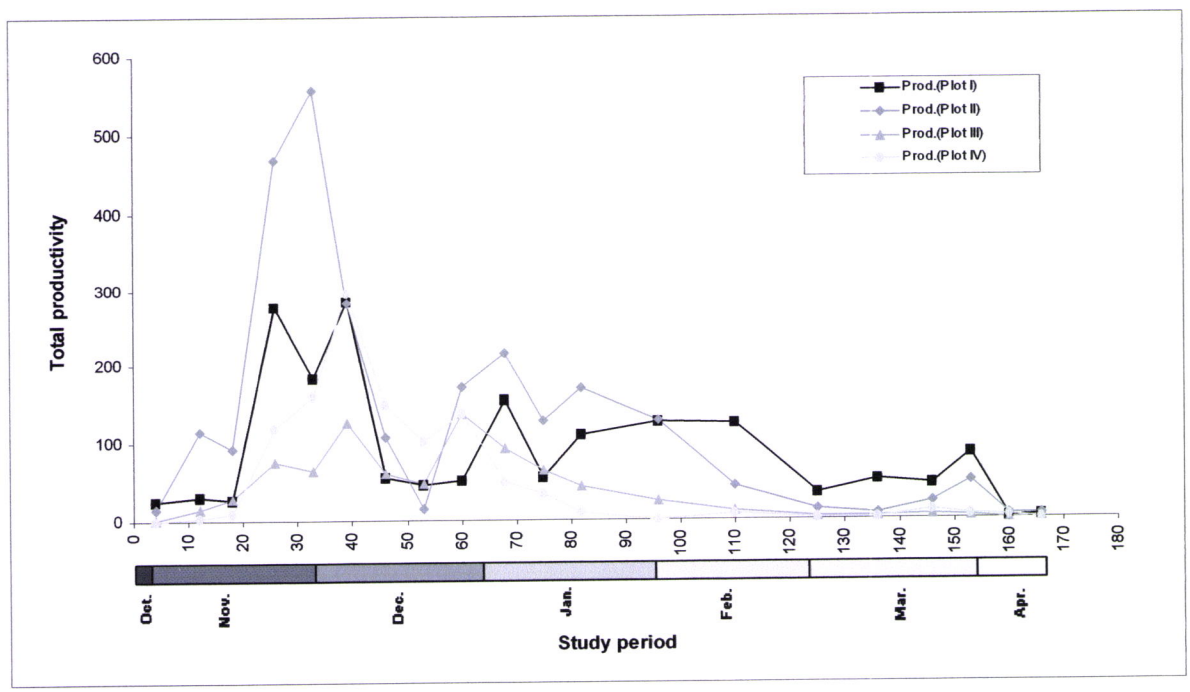
Species with the longest fruiting period, assessed by sporocarp presence during the study period, were: *Coprinus comatus* (90 %), *Melanoleuca exscissa var. exscissa* (71 %), *Panaeolus campanulatus* (62 %), *Clitocybe obsoleta* (62 %) and *Panaeolus acuminatus* (57 %) in plot I; *Coprinus comatus* (81 %), *Clitocybe obsoleta* (67 %), *Agaricus* sp. 1 (52 %), *Panaeolus campanulatus* (52 %) and *Panaeolus acuminatus* (52 %) in plot II; *Lepista flaccida* (52 %), *Laccaria laccata* (38 %), *Entoloma sericeum* (33 %), *Clitocybe gibba* (29 %) and *Macrolepiota konradii* (29 %) in plot III; *Laccaria laccata* (52 %), *Panaeolus acuminatus* (33 %) and *Agaricus* sp. 2 (29 %) in plot IV.

Species richness and productivity during the study period

Despite differences in the species list of each sampling plot, a phenological pattern similar to the entire macro-fungal community was observed through graphical analysis (Figure 5).



(1)



(2)

Figure 5. Species richness (1) and total productivity (2) variations during the study period, on each sampling plot.

In fact, variations in species richness and total productivity on each plot, during the study period, followed the same tendency, showing a maximum coincident peak between mid-November and early December, a second peak between late December and early January and a last, less intense peak in late March.

During this time, a significant decrease of both biological parameters was observed in mid-December and March.

This similar tendency was statistically tested; 4 % of the correlations were significant for $p < 0.05$ and 96 % were significant for $p < 0.01$, between species richness and total productivity of the four sampling plots and of the *montado* ecosystem.

The Friedman test revealed significant differences between sampling plots regarding the number of species. *A posteriori* STP tests grouped plots I-II and plots III-IV as similar (Table 5).

Table 5. Statistical results of the Friedman Test and *a posteriori* Simultaneous Tests Procedures (STP)

SAMPLING PLOT	NUMBER OF SPECIES		
	Mean	Median	25th-75th Percentiles
Plot I	6.1	6	4.0 – 7.5
Plot II	6.2	7	3.5 – 8.5
Plot III	3.4	3	1.0 – 5.0
Plot IV	4.1	4	1.0 – 6.5
Friedman Test	$\chi^2 = 32.611$; df = 3; $p < 0.001$		
A posteriori Simultaneous Tests Procedures (STP)	Plot I Plot II Plot IV Plot III		

Relations between trophic groups

Regarding the representativeness of each functional group, the following saprobic/mycorrhizal/parasitic percentages were observed: 94/ 3/ 3 in sample plot I, 76/ 24/ 0 in plot II, 71/ 25/ 4 in plot III and 67/ 30/ 3 in plot IV. During the study period, saprobic fungi species dominated almost exclusively.

IV. Relation between macro-fungal distribution and ecological parameters – spatial patterns

Confrontation of genera productivity with ecological parameters of each sampling plot by means of PCA allowed the detection of some tendencies (Figure 6).

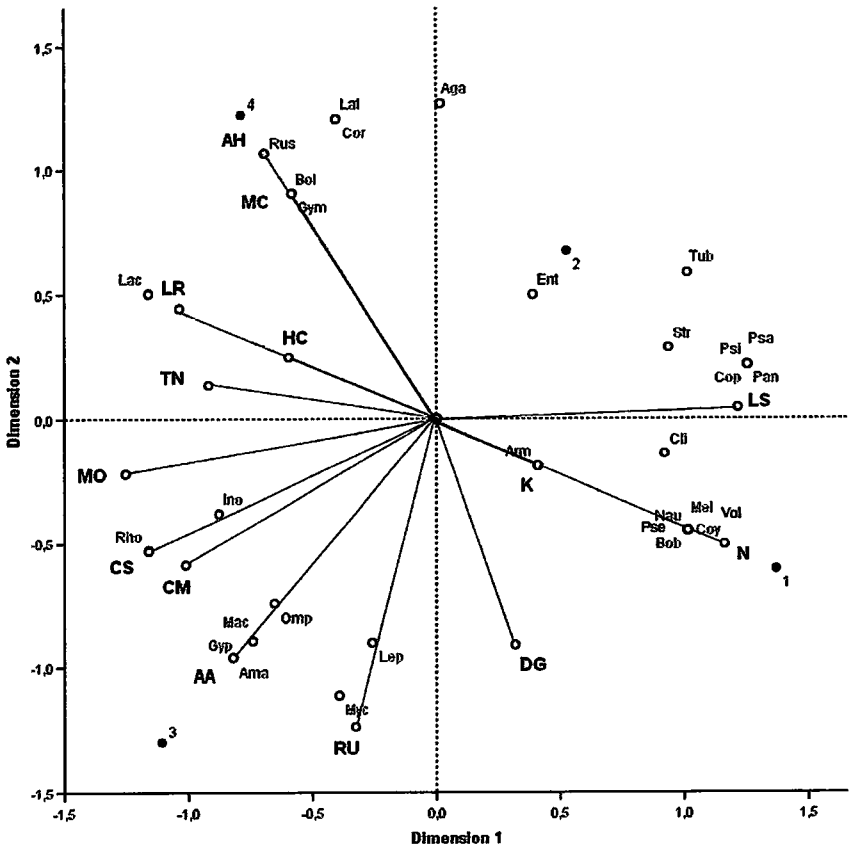


Figure 6. PCA ordination diagram of sampling plots, environmental factors and fungal genera based on sporocarp abundance (sampling plots: 1 - I, 2 - II, 3 - III, 4 - IV; environmental factors: AA - *Asparagus aphillus*, AH - medium tree height, CM - *Crataegus monogyna*, CS - *Cistus salviifolius*, DG - *Daphne gnidium*, HC - herbaceous cover, LR - litter, LS - livestock, MC - *Myrtus communis*, MO - organic matter, RU - *Rubus umifolius*, TN - tree number, K – potassium, N - nitrogen; Fungal genera: Aga – *Agaricus*, Ama – *Amanita*, Arm – *Armillaria*, Bob – *Bolbitius*, Bol – *Boletus*, Cli – *Clitocybe*, Coy – *Conocybe*, Cop – *Coprinus*, Cor – *Cortinarius*, Ent – *Entoloma*, Gym – *Gymnopilus*, Gyp – *Gymnopus*, Ino – *Inocybe*, Lac – *Laccaria*, Lat – *Lactarius*, Lep – *Lepista*, Mac – *Macrolepiota*, Mel – *Melanoleuca*, Myc – *Mycena*, Nau – *Naucoria*, Omp – Omphalina, Pan – *Panaeolus*, Psa – *Psathyrella*, Pse – *Pseudoclitocybe*, Psi – *Psilocybe*, Rho – *Rhodocollybia*, Rus – *Russula*, Str – *Stropharia*, Tub – *Tubaria*, Vol – *Volvariella*.

Axes 1 and 2 of the PCA graph explain, respectively, 46.9 % (eigenvalue 20.6) and 30.2 % (eigenvalue 13.3) of total data variability, which indicates that the two first dimensions explain 77.1 % of total variance. Sampling plots, represented by numbers 1 to 4, are displayed in a distinct quadrant, emphasizing the importance of particular micro-environmental features in distribution pattern and genera productivity.

The distribution of genera vectors indicates the existence of groups more related to a specific plot and, consequently, to corresponding biophysical parameters.

The first PCA axis reflects vegetation structural complexity, grazing intensity and ecological preference of genera: the left side of the diagram groups the sampling plots with higher vegetation cover and, therefore, with particular microclimatic and environmental conditions, such as lower light penetration, higher

moisture and greater thickness of the organic layer, concentrating most of the mycorrhizal genera (*Boletus*, *Cortinarius*, *Inocybe*, *Laccaria*, *Lactarius* and *Russula*) and some saprobic fungi associated with more forest-related areas; the right side of the diagram represents open areas, highly grazed and with a higher nitrogen content, concentrating genera related to nutrient-enriched areas (*Bolbitius*, *Coprinus*, *Panaeolus* and *Stropharia*).

Additionally, conjoint analysis of both axes allows a distinction between sampling plots III and IV according to composition, richness and cover of shrubs: placed in the top left quadrant are most mycorrhizal genera associated with medium shrub complexity, but with a higher number of potential host trees and thicker organic layer; the bottom left quadrant displays saprobic genera associated with high shrub complexity and less tree cover.

Discussion

I. Relation between species richness and productivity – phenological pattern

According to the results obtained, the fruiting period occurred from late October to early April. In a global analysis, a preferential fruiting period was apparent, in late November, when peaks of species richness and productivity maximums were reached. A second but less intense fruiting period also occurred in the beginning of spring.

On the other hand, a significant positive relation ($r = 0.89$; $p < 0.01$) was observed between sporocarp maximum production and species richness during the study period, which concurs with O'Dell *et al.* (1999), Straatsma *et al.* (2001), Straatsma & Krisai-Greilhuber (2003), Straatsma *et al.* (2003) and Bonet *et al.* (2004), indicating that most species show coincident phenology. Straatsma *et al.* (2001), Straatsma & Krisai-Greilhuber (2003) and Straatsma *et al.* (2003) evaluated total sporocarp and species variations in Switzerland and Austria over the years and concluded that species richness, generally, is a valuable parameter in expression of productivity.

Despite results that are not totally conclusive and statistically significant, comparative graphic analysis between species richness and productivity variations and variations in abiotic parameters during the study period suggests that biological parameters react to the fluctuation of physical parameters, for a certain period of time.

The importance of physical parameters, especially rainfall, on the fruiting process has been pointed out in several studies (Bills *et al.* 1986; O'Dell *et al.* 1999; Straatsma *et al.* 2001; Salerni *et al.* 2002; Giachini *et al.* 2004). However, comparison of diverse studies (Bills *et al.* 1986; Brunner *et al.* 1992; Matsuda & Hijii 1998; Schmit *et al.* 1999) shows that this pattern varies with latitude, since it is dependent on climatic conditions. So, as a result of variation of climatic conditions proper to each geographical region, an adaptation of the phenological pattern occurs.

In the present study, fruiting period was certainly conditioned by the characteristic Mediterranean climate of this region. Nonetheless, for the same location, fluctuations may occur in fruiting peak and period that are probably related to annual variation of climatic parameters (Perini *et al.* 1996).

Salerni *et al.* (2002) studied the effects of Mediterranean climate (minimum, mean and maximum rainfall and temperature) in macro-fungal fruiting in oak forests in Tuscany, Italy, and concluded that the total number of species and sporocarps were correlated with annual and seasonal rainfall and temperature, showing a fruiting pattern similar to that observed in the present study. In the same study, Salerni *et al.* (2002) also observed a significantly positive correlation between rainfall and species diversity and a less significant correlation between this physical parameter and sporocarp abundance. This means that, in oak woods, rainfall necessary to induce fruiting is similar for all species, but different species react distinctly in terms of the number of sporocarps produced, according to direct dependence of mycelium metabolic activity on soil and moisture level.

Variations in fruiting process thus seem to depend on a particular balance between mean temperature and total rainfall.

II. Species richness and relation between trophic groups: importance of environmental and ecological characteristics

During the present study, 59 species were identified, included in 30 genera, of which 45 (76 %), 13 (22 %) and 1 (2 %) are, respectively, saprobic, mycorrhizal and parasitic.

Species richness was low when compared with other studies involving sporocarp sampling and considering total macro-fungi, whether in conifer or hardwood forests (Villeneuve *et al.* 1989; Brunner *et al.* 1992; Schmit *et al.* 1999; Richard *et al.* 2004; Trudell & Edmonds 2004) (Table 6).

Table 6. Total species richness of macro-fungal communities in previous studies performed in conifer, mixed and hardwood forests

DOMINANT HOST TREE	CONTINENT; COUNTRY	TOTAL SPECIES RICHNESS	REFERENCE
Conifer Forests			
Picea abies	Europe; Sweden	48 ECM fungi	Dahlberg *et al.* (1997)
Picea abies	Europe; Switzerland	128 ECM fungi	Peter *et al.* (2001)b
Pinus sylvestris	Europe; Spain	164 ECM fungi	Bonet *et al.* (2004)
Tsuga heterophylla-Pseudotsuga menziesii	America; U.S.A.	150 ECM fungi	O'Dell *et al.* (1999)
Pseudotsuga menziesii	America; U.S.A.	263 ECM fungi	Smith *et al.* (2002)
Pseudotsuga menzierii, Tsuga heterophylla, Picea sitchensis, Thuja plicata	America; U.S.A.	214 species (ECM and saprobic fungi)	Trudell & Edmonds (2004)
Abies firma	Asia; Japan	39 ECM fungi	Matsuda & Hijii (1998)
Mixed Forests			
Betula, Acer, Abies, Picea	America; Canada	195 species (ECM and saprobic fungi)	Villeneuve *et al.* 1989
Quercus rubra and *Tsuga canadensis*	America; U.S.A.	67 ECM fungi	Walker & Miller (2002)
Pinus taeda and *Eucalyptus dunni*	America; Brazil	34 ECM fungi	Giachini *et al.* (2004)
Hardwood Forests			
Quercus ilex	Europe; Corsica Island	234 species (166 ECM and 68 saprobic fungi)	Richard *et al.*, 2004
Alnus tenuifolia e *Alnus crispa*	America; U.S.A.	131 species	Brunner *et al.* (1992)
Quercus spp.	America; U.S.A.	174 species (36 ECM and 138 saprobic fungi)	Schmit *et al.* (1999)
Eucalyptus globulus	Oceania; Australia	30 ECM fungi	Lu *et al.* (1999)

Generally, differences in species richness between several studies may be related to methodological differences (stand age, sampling period, sampling plot dimensions) (Bills *et al.* 1986; O'Dell *et al.* 1999; Schmit *et al.* 1999), macroclimatic differences inherent to the latitude/altitude gradient (Laganà *et al.* 1999; Laganà *et al.* 2001) and differences in complexity, structure and composition of the ecosystem vegetation, that condition the available ecological niches and the system dynamics (Bills *et al.* 1986; Villeneuve *et al.* 1989).

The reduced species richness in the area studied could be associated with limitations intrinsic to sampling method and period. In fact, since this study was developed during 1 year and exclusively considered sporocarp sampling to evaluate the macro-fungal community, it was conditioned to fruiting process unpredictability, which reacts to weather fluctuations, and dependent on the species phenological pattern.

Works by Bills *et al.* (1986), Dahlberg *et al.* (1997), O'Dell *et al.* (1999), Straatsma *et al.* (2001), Straatsma & Krisai-Greilhuber (2003) and Bonet *et al.* (2004), performed with different periods of time, showed an inter-annual variation in the composition, number and productivity of species.

Furthermore, the reduced species richness might result from disadvantageous conditions imposed by interactions between climate conditions during the study period and spatial arrangement of this *montado* ecosystem.

Landscape simplification and homogenization, as consequences of tree cover reduction, absence or low representativeness of shrub storey and intense grazing, confer specific ecological and microclimatic characteristics that may be less favourable to macro-fungi.

However, low species diversity in this particular case is not related to the host tree, since Laganà *et al.* (2001) obtained higher values of this parameter in evergreen *Quercus* spp. woods in the Mediterranean region. These differences may be related to the system exploitation and management practices, particularly to bovine livestock presence, which causes changes in herbaceous and shrub vegetation and soil compactness.

On the other hand, work developed by Richard *et al.* (2004) in a similar Mediterranean ecosystem, dominated by *Quercus ilex*, showed a relation between trophic groups different from the one observed in the present study, with a dominance of EMC (166) over saprobic species (68). These results suggest that the proportion between trophic groups is not exclusive to an ecosystem but reflects particular micro-environmental characteristics which model the macro-fungal communities, favouring one group or another according to its ecological needs. Indeed, the symbiotic nature of ECM species imposes a strict or a more generalist dependence on the host tree while saprobic species are, generally, more related to occasional or constant presence of favourable substrata. This indicates that the low representativeness of ECM species in the studied macro-fungal community, in number and proportion, when compared with other works (Dahlberg *et al.* 1997; Matsuda & Hijii 1998; Lu *et al.* 1999; O'Dell *et al.* 1999; Peter *et al.* 2001b; Smith *et al.* 2002; Walker & Miller 2002; Bonet *et al.* 2004; Giachini *et al.* 2004; Richard *et al.* 2004) probably results from interaction between the host symbiotic nature and more unfavourable environmental and ecological conditions for the colonization process and/or fruiting.

However, the reduced sporocarp productivity of ECM fungi does not necessarily mean a below-ground low representativeness or absence. The study developed by Azul (2002) on the below-ground ECM community in *Quercus suber montado*, in Portugal, revealed high species diversity despite the low sporocarp diversity.

The generalized dominance of saprobic species might be due to intense grazing. In fact, bovine livestock provides considerable amounts of excrement which constitute direct nutrition substrata to several saprobic species found on the sampling plots. Simultaneously, excrement integration in the organic matter soil layer supplies additional nitrogen content favourable to other saprobic species.

This tendency was also demonstrated by Pinho-Almeida & Ferreira (2005) in a similar ecosystem.

Moreover, saprobic species (*Coprinus comatus*, *Psathyrella hirta*, *Lepista flaccida*, *Panaeolus acuminatus*, *Panaeolus campanulatus* e *Clitocybe obsoleta*) contributed with higher sporocarp production during the fruiting peak, considering all the sampling plots. These species presence and abundance reflect habitat peculiarities, since more than half of the most productive saprobic species are coprophilous. On the other hand, the only mycorrhizal species that produced more sporocarps, *Laccaria laccata*, is referred by some authors (Bills *et al.* 1986; Laganà *et al.* 2001; Giachini *et al.* 2004; Richard *et al.* 2004) as one of the most abundant ECM species, which suggests a more widespread ecological range and lower environmental requirements and host specificity.

The higher representativeness of *Agaricaceae* and *Tricholomataceae*, considering all species present in the four sampling plots, was not conclusive when compared with previous works developed in evergreen hardwood forests (Azul 2002; Richard *et al.* 2004), but emphasizes the importance of study area particularities and might contribute to more detailed characterization of each Family.

The analysis of each sampling plot revealed distinct total species richness and proportions between trophic groups: 31 species (94 % saprobic, 3 % ECM and 3 % parasitic) in plot I; 21 species (76 % saprobic and 24 % ECM) in plot II; 24 species (71 % saprobic, 25 % ECM and 4 % parasitic) in plot III; and 30 species (67 % saprobic, 30 % ECM and 3 % parasitic) in plot IV. Furthermore, a reduced number of shared species was observed among sampling plots (8 %), as well as a considerable number of exclusive species (52 %) and a higher similarity between plots I-II and plots III-IV. Indeed, despite this study having only focused on a single fruiting period, only partially representing this area's mycological potential, it allows the identification of the possible micro-environmental factors that influence the macro-fungal community, excluding the macro-environmental parameters that equally condition the sampling plots.

Vegetation structure and density

Comparison of vegetation structure, composition and stratification of the four sampling plots revealed low tree numbers and reduced shrub and tree cover in plots I and II, whereas plots III and IV included a higher number of hosts, either trees or shrubs.

An increase in forest complexity from plots I and II to plots III and IV was accompanied by an increase in tree number and canopy density, which resulted in decreased light and wind penetration, variation of air and soil temperature and humidity and increased litter and organic matter. This interaction among environmental factors is of great importance in establishing favourable conditions to certain forest and specific ECM fungi (Bonet *et al.* 2004). In the present study, the complexity gradient resulted in species richness decrease.

The negative effect on fungal species richness of tree density and number of vegetation layers increase was demonstrated by Richard *et al.* (2004).

Nonetheless, considering the different trophic groups, a significant increase in ECM species and a saprobic species decline was observed with canopy closure. Besides, the higher percentage of ECM fungi observed in plot IV may be related to the presence of regenerating Mediterranean shrub, particularly *Cistus salviifolius*. In general, *Cistus* constitute potential hosts to several mycorrhizal species (Comandini *et al.* 2006).

The positive relation between ECM species richness and tree cover density is contrary to the study by Richard *et al.* (2004), but concurs with Villeneuve *et al.* (1989) and Laganà *et al.* (1999). Villeneuve *et al.* (1989) evidenced a significant positive correlation between ECM species and host tree cover ($r = 0.50$), suggesting that the latter parameter, related with root surface and available energy, may influence the number of ECM fungi on a site. On the other hand, Laganà *et al.* (1999) observed, in Mediterranean forest ecosystems, a significant positive correlation between ECM species and tree species ($r = 0.48$; $p < 0.01$) and tree cover ($r = 0.43$; $p < 0.001$ for a cover > 50 %).

Moreover, Azul (2002) verified that ECM colonization and species richness are directly related with understorey presence of underdeveloped shrub cover (25 – 30 %).

Edaphic conditions

Besides system structure and complexity, the four sampling plots presented distinct edaphic conditions, which appear to influence species colonization and fruiting processes.

Plots I and II showed higher contents of assimilable nitrogen and phosphorus and a lower percentage of organic matter, when compared with plots III and IV. This result is in harmony with saprobe species abundance in each plot, since reduced organic matter content, together with a higher percentage of nutrients available in the soil, reflect an active and efficient process of decay.

Soil potassium levels are not very conclusive, since this element is rapidly leached and released, as a result of its weak structural relation with organic matter (Mason 1976).

On the other hand, distinct shrub and tree cover and grazing intensity between plots I-II and III-IV result in different nutrient inputs: plots I-II showed a reduced or almost absent litter layer but a higher and constant presence of livestock; plot III and, especially, plot IV showed more consistent organic layer and very occasional presence of livestock.

Organic debris from bovine livestock represents a source of easily and rapidly assimilable nutrients, mainly nitrogen. Substrata rich in organic debris will certainly be more attractive to saprobic species but also to vascular species existing in the system. In this way, hosts will not depend so strictly on symbiotic relations for subsistence. Under these circumstances and considering that the absence of sporocarps of ECM species does not represent a conclusive indication of its below-ground absence, it is probable that the ECM community is, in this ecosystem, reduced or almost inexistent.

The importance of edaphic structure to colonization and fruiting processes has been demonstrated in several studies (e.g. Villeneuve *et al.* 1989; Kernaghan & Harper 2001; Egli & Ayer 2003) and, especially, the particular effect of nitrogen. In fact, most studies confirm the existence of a probable cause-effect relation between nitrogen increase (mainly in its mineral form) inherent to air pollution deposition and fertilization and the diminishment of ECM species fruiting (e.g. Arnolds 1991; Lilleskov *et al.* 2001; Peter *et al.* 2001a; Avis *et al.* 2003).

The means by which nitrogen affects ECM fungi seems to be related with carbon allocation (Trudell and Edmonds 2004). However, nitrogen increase, besides interfering in host-fungi relations, causes alterations in pH, available base cations and heavy metals (Lilleskov & Bruns 2001; Lilleskov *et al.* 2001).

Lilleskov *et al.* (2001) assessed nitrogen deposition effects along an abundance gradient on ECM communities of conifer forests (*Picea glauca*) and observed lower species richness and sporocarp abundance in areas with higher mineral nitrogen content. In parallel, results revealed that the environmental variables more correlated with macro-fungal species occurrence were organic horizon mineral nitrogen, organic horizon net mineralization and mineral soil base cations.

Peter *et al.* (2001a) evaluated the influence of nitrogen addition, during a 2-year period, on sporocarp production of ECM and saprobic species and on below-ground ECM species composition in a conifer forest (*Picea abies*). Sporocarp sampling revealed a drastic reduction of ECM fungi diversity after one year of nitrogen addition while the saprobic community was not affected. Impact on below-ground ECM fungi diversity was less intense, without alterations in the number of ECM species but with shifts in composition and abundance of species. The study shows that nitrogen increase may influence mycorrhizal establishment, production and distribution of extra-radical mycelium and sporocarp formation; however, mycorrhizal colonization appears to be less sensitive to nitrogen input increase than sporocarp formation.

Moreover, Avis *et al.* (2003) tested the fertilization influence on ECM fungi for three years in a temperate *Quercus* ecosystem and observed a decrease of sporocarp diversity, especially *Amanita*, *Boletus* and *Cortinarius* spp. and an alteration of the community composition in fertilized plots. Nevertheless, an increase of *Russula* spp. community was observed with a nitrogen addition of 17 $g.m^{-2}.year^{-1}$.

In less disturbed ecosystems, nitrogen content influences, in the same way, proportions between trophic groups in macro-fungal communities, favouring saprobic in detriment of ECM fungi.

Brunner *et al.* (1992), related different abundances of ECM and saprobic fungi with high nitrogen soil content associated with Alder forests (*Alnus tenuifolia* and *Alnus crispa*) suggesting that the low percentage of ECM species might be due to their high sensitivity to nitrogen levels and the superior saprobic species richness, mainly lignicolous, could be a direct result of dominance of dead organic substrata.

Furthermore, Trudell & Edmonds (2004) characterized and compared the epigeous fungal community in two mature conifer forests, with minimum anthropogenic interference, in order to evaluate the importance of humidity and nitrogen abundance to macro-fungal diversity. They verified that, regardless of similarity between dominant tree species and macro-fungal species richness, the communities were very distinct insofar as the most representative genera, trophic group proportions and productivity: the driest and nitrogen-poor area showed a higher sporocarp productivity and dominance of *Cortinarius*, *Tricholoma*, *Hydnellum*, *Suillus* and *Sarcodon* genera; the wetter and nitrogen-rich area revealed lower productivity and dominance of *Inocybe*, *Russula*, *Amanita*, *Boletus* and *Phaeocollybia*, as well as a significant percentage of saprobic fungi. Taxonomic differences between the two areas appear to be related to differences in eco-physiological characteristics, especially in mycelium morphology and sensitivity to soil moisture and mineral nitrogen abundance. Nitrogen and soil moisture seem to be the most determinant

factors in differences among macro-fungal communities. Therefore, in this study, despite its importance, host specificity is not the dominant factor, since there were few shared species between the two areas; of the 214 identified species, only 33 (15 %) appeared in both areas, 16 ECM and 17 saprobic.

The influence of nitrogen and edaphic structure on macro-fungal communities was also shown by Baar (1996), who assessed ECM species composition and sporocarp abundance in primary and secondary *Pinus sylvestris* stands. The author observed that species richness and diversity were superior in primary stands or stands with removal of the organic layer (litter, humus and herbaceous vegetation), concluding that simultaneous alteration of tree physiology and soil chemical composition, inherent to forest maturing processes, influences ECM fungi communities. In this study, low nitrogen concentrations in ectorganic layers and in mineral soil were more propitious to the establishment of ECM mycelium.

Conclusions

The present study represents an important contribution to knowledge of the macro-fungal community that colonizes the *montado* ecosystem, although focused on only one year.

Analysis of the results demonstrated that particular ecosystem characteristics, imposed by vegetation spatial structure and more or less constant livestock presence, influenced macro-fungal communities. Dominance of saprobic species may have been enhanced by conjugation of these factors and, mostly, by soil nitrogen availability.

Nonetheless, differences between species identified in the four sampling plots, in a particular period of time and having the same macro-environmental conditions, probably reflected micro-environmental differences associated with distinct management strategies.

In the most open and grazed areas (plots I and II) a higher number of species and greater productivity was observed, relative to denser and less grazed areas (plots III and IV). These differences are even more evident when representativeness of each trophic group is considered.

Most species that contribute to the significant increase of productivity in plots I and II are saprobic, appearing associated with occasional substrata related with the presence of livestock. ECM species appear, preferentially, in areas with higher ecological complexity and host density, plots III and IV. However, reduced fructification of ECM fungi is not conclusive, given the unpredictability of this process that depends on exogenous, mainly environmental, and endogenous factors. Regardless of the fragility of

the results obtained, most studies concur with these observations. They describe reduction of ECM species fructification or, inclusively, a below-ground species replacement or disappearance, with the increase of soil nitrogen, either by atmospheric deposition or fertilization (e.g. Lilleskov *et al.* 2001; Peter *et al.* 2001a; Avis *et al.* 2003) and increased ECM fungi diversity in areas with higher density of potential tree hosts and medium complexity of vegetation layers (Villeneuve *et al.* 1989; Laganà *et al.* 1999; Azul 2002).

In addition to impacts on ECM communities, high nitrogen availability also implies negative effects on plant biodiversity and ecosystem function (Vitousek *et al.* 1997). On the other hand, this ecosystem showed some signs of debility through the presence of a parasitic species on three sampling plots.

The short duration of the present work also limits the concrete definition of strategies and management measures for sustainable ecosystem exploitation. However, any plan targeted to higher economic profitability while respecting the ecosystem balance should favour colonization and fructification of macro-fungal communities. Saprobe species assume a fundamental role in organic matter turnover and, inclusively, some species develop edible sporocarps which constitute a resource with potential economic value. ECM species contribute to increased host establishment, helping the host to subsist in adverse conditions and protecting it from pathogenic agents. Also, several ECM species invest in sporocarps with high economic and gastronomic value, difficult to obtain in *ex situ* cultures.

Considering the importance of the main functional groups present in this ecosystem, management should be more aimed at favouring ECM species, since host protection and health would also be assured, maximizing the quality of its sub-products (cork and acorn). On the other hand, ECM species require more effective protection, given their vulnerability to anthropogenic activities in the ecosystem and their generalized decline in several other countries.

Wiensczyk *et al.* (2002) suggest some management practices to maintain ECM community diversity. However, for the studied ecosystem, the most adequate management measures should include:

- Reduction of grazing intensity and, if bovine livestock maintenance is intended, diminishment of cattle head, in order to decrease input of nitrogen-rich organic debris, soil compaction inherent to excessive livestock treading, and vegetation degradation;
- Avoidance of soil tilling with heavy machinery, since it could destroy macro-fungal mycelium;
- Avoidance of soil fertilization, since it constitutes an additional nitrogen input which will interfere in ecosystem balance, particularly in host-ECM species relations;

- Maintenance of high density of tree cover, assuring higher availability of ecological niches to ECM species;
- Maintenance of some shrub under-storey areas of the Mediterranean-type *maquis*. According to Azul (2002), *montado* ecosystems, as extensive production systems, should include areas with shrub under-storey constituted by autochthonous species, with a cover percentage of 25 - 30 % and 1 m of maximum height;
- Avoidance of excessive sporocarp collection, since it could interfere in reproduction and, as a consequence, in the species perpetuation in this ecosystem.

Sustainable exploitation of this ecosystem will certainly be more advantageous to the communities equilibrium and will permit a higher economic valuation. In addition to all the sub-products associated with the cork oak, edible sporocarp collection will constitute an extra gain.

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III. Considerações Finais

O reconhecimento do valor ecológico e económico dos fungos e a constatação de uma redução de espécies ECM face aos impactes associados ao desenvolvimento industrial, tem incentivado o desenvolvimento de inúmeros estudos, contribuindo para o aumento do conhecimento da diversidade da micobiota e da biologia, ecologia, distribuição e estatuto de ameaça de cada espécie em particular.

No entanto, são relativamente poucos os estudos desenvolvidos, neste domínio, na Região Mediterrânica (e.g Perini *et al.* 1996; Laganà *et al.* 1999; Laganà *et al.* 2001; Salerni *et al.* 2002; Bonet *et al.* 2004; Richard *et al.* 2004; Ortega & Esteve-Raventós 2005; Ortega & Navarro 2006) e ainda mais escassos em Portugal, desconhecendo-se a diversidade e os estatutos de ameaça das espécies, o que dificulta a definição de planos de protecção ou gestão dos ecossistemas.

Nesse sentido, o presente estudo pretendeu contribuir para o aumento do conhecimento da diversidade de macrofungos em Portugal e, particularmente, num ecossistema dominante da paisagem do Sul - montado de sobreiro - , que se encontra numa situação crítica, em resultado da implementação de medidas de gestão desadequadas. O investimento na recuperação deste ecossistema é fundamental, na medida em que para além de incluir uma diversidade de espécies superior à comunidade "clímax" onde se desenvolve o sobreiro, integrando espécies características de bosques e de prados e pastagens, representa um exemplo de um sistema humanizado ecologicamente sustentável (Azul 2002; Díaz *et al.* 2003).

De facto, os sistemas de montado são considerados ecossistemas complexos, em equilíbrio, que constituem uma etapa de sucessão ecológica induzida, requerendo uma intervenção antrópica constante (Scarascia-Mugnozza *et al.* 2000). No entanto, as intervenções mais ou menos intensas, através do corte da vegetação, interferência nas camadas orgânica e mineral do solo e/ou introdução de gado, podem conduzir a um desequilíbrio e alteração estrutural das comunidades micobióticas do solo ou, inclusivamente, a uma degradação generalizada de todo o sistema (Courtecuisse 2001; Pilz & Molina 2001; Wiensczyk *et al.* 2002). A predominância e/ou eliminação de determinadas espécies de fungos, dependerá da sua capacidade de dispersão e da especificidade relativamente ao hospedeiro (Azul 2002).

A avaliação simultânea da potencialidade micológica neste sistema e das condições microambientais que favoreceram a colonização e frutificação de macrofungos e especificamente das espécies ECM permitiram definir algumas estratégias de gestão que, certamente, poderão contribuir para a regressão deste tendência, valorizando a protecção desta comunidade e maximizando a disponibilidade e qualidade de recursos naturais.

Este estudo evidenciou a presença de uma importante comunidade de macrofungos associada ao ecossistema de montado, dominada por espécies sapróbias. Esta dominância, associada a um número de espécies relativamente baixo em comparação com outros estudos desenvolvidos em sistemas mediterrânicos dominados por *Quercus* spp. (e.g. Laganà *et al.* 2001; Richard *et al.* 2004), pareceu estar relacionada com a configuração espacial do ecossistema e presença, mais ou menos intensa, de gado bovino, que contribuiu, significativamente, para a compactação e enriquecimento de azoto no solo.

A maior representatividade de espécies sapróbias aponta para possíveis sinais de fragilidade no ecossistema em estudo, na medida em que apesar destas espécies desempenharem uma função ecológica fundamental, contribuindo para o *turnover* dos nutrientes e investirem em carpóforos altamente rentáveis, não asseguram o equilíbrio de ecossistema. Por outro lado, o excesso de azoto, no solo ou substratos ocasionais, que favoreceu a presença e frutificação destas espécies, não é benéfico para as espécies vegetais presentes. Esta debilidade do ecossistema foi ainda reforçada pela presença de uma espécie parasita em três parcelas de amostragem.

Adicionalmente, a comparação entre a composição de espécies e a proporção de grupos tróficos entre as parcelas de amostragem evidenciou a influência das características microambientais no processo de colonização e frutificação e, particularmente, da complexidade estrutural da vegetação e pressão do pastoreio: as áreas mais abertas, sujeitas a uma maior pressão do pastoreio (I e II), revelaram-se mais favoráveis aos fungos sapróbios; as áreas mais fechadas e complexas (III e IV), no que respeita aos estratos arbóreo e arbustivo, e com menor intensidade de pastoreio pareceram mais favoráveis às espécies micorrízicas.

Apesar da fragilidade dos resultados, inerentes às limitações metodológicas, corroboraram a maioria dos estudos, demonstrando a influência negativa do azoto nas comunidades de fungos ECM e neutra ou positiva nas comunidades de fungos sapróbios (e. g. Arnolds 1991; Lilleskov *et al.* 2001; Peter *et al.* 2001a; Avis *et al.* 2003).

Considerando a importância ecológica dos fungos ECM em qualquer ecossistema, a maior valorização dos carpóforos produzidos por estas espécies e o declínio registado, nos últimos anos, na Europa, será mais vantajoso direccionar as estratégias de gestão para o favorecimento deste grupo trófico.

Wiensczyk *et al.* (2002) sugere algumas práticas de gestão que visam a manutenção da diversidade da comunidade de espécies ECM. No entanto, para o ecossistema em estudo, deverão considerar-se as seguintes medidas de gestão:

1. Reduzir a intensidade do pastoreio e, no caso de se pretender manter o gado bovino, diminuir o encabeçamento de forma a reduzir o *input* de detritos orgânicos ricos em azoto no solo, a compactação do solo inerente ao pisoteio excessivo e a degradação da vegetação. De facto, os encabeçamentos actuais desta área excedem significativamente o encabeçamento máximo de 2 Cabeças Normais por hectare definido pelas Boas Práticas Agrícolas (Artº 89 e Anexo VIII da Portaria nº 1212/2003).

2. Evitar o revolvimento e compactação do solo com maquinaria pesada, na medida em que poderá provocar uma destruição do micélio dos fungos;

3. Evitar a fertilização do solo, pois constitui um acréscimo de azoto que irá alterar o equilíbrio do sistema e particularmente a relação entre o hospedeiro e a espécies micorrízica;

4. Manter uma maior densidade arbórea, assegurando uma maior disponibilidade de nichos ecológicos para as espécies micorrízicas;

5. Manter algumas manchas de subcoberto arbustivo de mato mediterrânico. De acordo com Azul (2002), os ecossistemas de montado, como sistemas de produção extensivos, deverão incluir áreas com o subcoberto arbustivo constituído por espécies autóctones, com um índice de cobertura de 25 a 30 % e altura máxima de 1 m;

6. Evitar uma recolha excessiva de carpóforos, uma que poderá interferir na reprodução e, consequentemente, na perpetuação da espécie nesse ecossistema.

A gestão sustentada do ecossistema, que valorize o equilíbrio e a presença das comunidades de fungos ECM, será mais vantajosa ecologica e economicamente, na medida em que favorece a fitossanidade do sistema, maximiza a qualidade dos sub-produtos do sobreiro (cortiça e bolota) e introduz um novo recurso natural altamente rentável: os cogumelos comestíveis.

Em Portugal, apesar de existir uma tradição de colheita de algumas espécies de cogumelos comestíveis para auto-consumo, intrínseca a cada região, persiste um sentimento de micofobia motivado pelos inúmeros casos de envenenamento relatados todos os anos. No entanto, sabe-se que existe uma actividade de colheita paralela, mais intensa, com vista à comercialização e, principalmente, exportação para os mercados mundiais, incentivada pela valorização económica deste recurso e pela inexistência de legislação. Apesar de se desconhecer a verdadeira dimensão desta actividade, crê-se que está em expansão e que será particularmente desvantajosa para os ecossistemas que sofrem os efeitos

inerentes à pressão desregrada da colheita e para os proprietários, que não usufruem desta mais-valia acrescida (Azul 2002).

A informação e sensibilização do público em geral e, particularmente, dos proprietários para a valorização e exploração deste recurso alternativo, numa perspectiva sustentável, contribuirão, igualmente, para a protecção da biodiversidade dos macrofungos e dos ecossistemas.

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