CLÁUDIA PATRÍCIA TRINDADE CRUZ

Epiphytic lichens as surrogates for biodiversity and ecosystem resilience in native oakwoods of the Baixo Tâmega region (Northern Portugal)

Departamento de Botânica
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December 2009

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Thesis submitted to Faculdade de Ciências da Universidade do Porto
to obtain the degree of Master of Biology

Thesis supervised by Prof. João Honrado

Departamento de Botânica
Faculdade de Ciências da Universidade do Porto
December 2009

To my parents and brother

“Lichens are fungi that have discovered agriculture.”
TREVOR GOWARD

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ABSTRACT

In the last 50 years, concern about the loss of lichen diversity associated with forest management and forest fragmentation has led to many studies designed to assess patterns and monitor trends of lichen biodiversity in forests worldwide. However there are surprisingly few studies focusing on the effect of natural disturbance for epiphytic lichens in forest ecosystems and, especially, on how these changes affect the relationships between epiphytic lichens and other organisms. The major goal of this thesis was to characterize and valuate the epiphytic lichens in the Baixo Tâmega region (northern Portugal) and to assess its vulnerability to several drivers of change, particularly fire.

The study area is located in Aboboreira and Castelo, a mountain area with 105 km^2 and a top altitude of approximately 1000 m.a.s.l. In this region, fire is one of the worst natural catastrophes not only because of its high frequency and wide extension but also because of their enormous destructive effects.

This thesis has included three case studies that proved the need to develop management and conservation actions for the area. In the first study we assessed the epiphytic lichen diversity in the oak woods of the Aboboreira and Castelo mountains. Results have revealed high diversity value and presence of a lichen community that has suffered general decline throughout Europe. The second study has addressed the impact of fire over epiphytic lichens and community recovering patterns. Results observed along the fire gradient showed that the most common and abundant lichen species establish themselves early in post-fire gradient while “old-growth” associated lichens tend to recover slowly along the same gradient. The third study intended to evaluate the potential use of selected lichen species or groups as biodiversity surrogates, in a scenario of fire-controlled ecological changes.

Results showed that the use of epiphytic lichens as surrogates of bryophyte and vascular plant diversity should consider not only species richeness and composition, but also occurrence of disturbing factors, such fire. among the studied groups, lichen genera is the only group that can be used as surrogate of total epiphytic lichen diversity, independently of fire induced changes.

RESUMO

Nos últimos 50 anos, o reconhecimento da perda de diversidade liquénica associada a práticas de gestão e fragmentação florestal conduziu a um aumento do número de estudos com o objectivo de monitorizar a diversidade liquénica das florestas a nível global. Contudo, o número de estudos sobre o efeito de perturbações naturais sobre os líquenes epifiticos é ainda bastante reduzido. A lacuna é especialmente evidente quando consideradas as relações entre a diversidade de líquenes epifíticos e a dos restantes organismos.

O principal objectivo desta tese foi caracterizar e valorar a diversidade de líquenes epifíticos da região do Baixo Tâmega (norte de Portugal) e determinar a sua vulnerabilidade a diversos factores de mudança, tais como o fogo.

A área de estudo localizou-se na Serra de Aboboreira e Castelo, uma área montanhosa com 105 km^2 e com altitude máxima de 1000 m. Nesta região, o fogo é uma das piores catástrofes naturais, não só devido à sua frequência e grande extensão como também ao seu enorme poder destrutivo.

Esta tese incluiu três estudos de caso que comprovaram a necessidade de desenvolver estratégias de gestão e conservação para a área em causa, no sentido da sua valorização. O primeiro caso constituiu um estudo da diversidade liquénica das florestas de carvalhos da Serra da Aboboreira e Castelo. Os resultados revelaram um valor de diversidade elevado e a presença de comunidades liquénicas que se encontram em declínio acentuado por toda a Europa. No segundo caso analisou-se o impacto do fogo sobre a diversidade de líquenes epifíticos e os padrões de recuperação pós-fogo destas comunidades liquénicas. Os resultados revelaram que as espécies mais comuns estabeleceram-se desde logo nas áreas ardidas e que estão em todas as fases de recuperação pós-fogo. Espécies indicadoras de estabilidade estrutural das florestas apresentaram uma das recuperações mais significativas ao longo do gradiente, atingindo a maior riqueza específica nas florestas não ardidas. O terceiro caso permitiu avaliar a potencialidade do uso de espécies de líquenes ou grupos liquénicos como indicadores, num cenário de elevada incidência de fogos florestais. Os resultados demonstraram que a utilização de líquenes epifíticos como indicadores da diversidade de briófitas e plantas vasculares, deve ter em conta não só a riqueza específica, mas também a composição das comunidades, bem como os factores de perturbação dos habitats florestais. Entre os grupos estudados, apenas os géneros liquénicos estavam significativa e altamente correlacionados com a diversidade liquénica, independentemente das alterações induzidas pelo fogo.

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1. INTRODUCTION

1.1 SCOPE, AIMS AND THESIS ORGANIZATION

This study was conducted within the framework of a wider ongoing project aimed at promoting the natural and cultural heritages as factors for sustainable development in the Baixo Tâmega region (Northern Portugal) and at assessing their relative vulnerability to several drivers of change (Honrado *et al.,* 2009). One of main goals of the mentioned project is the characterization and valuation of the natural heritage and resources of the territory, which includes the lichen flora.

The Aboboreira and Castelo mountains, located at the core of the Baixo Tâmega area, are known for the widespread regeneration of young oak woods as a result of agricultural and pastoral abandonment. However, in late-summer 2009, large wildfires occurred throughout the area, stressing the importance of fire as a control of vegetation and biodiversity. In this context, this study has three major goals: (1) to assess the epiphytic lichen diversity in the oak woods of the Aboboreira and Castelo mountains, (2) to examine the impact of fire over epiphytic lichens and community recovering patterns, and (3) to evaluate the potential use of selected lichen species or groups as biodiversity indicators, in a scenario of fire-controlled ecological changes.

This thesis includes results from three different surveys conducted in the Aboboreira and Castelo mountains. Overall, these surveys were performed to examine epiphytic lichen species richness, distribution patterns, and responses to disturbance along a post-fire gradient.

According to our objectives, the thesis was organized in three articles, one for each major goal.

1.2 A BIRD'S-EYE-VIEW OF FOREST LICHEN ECOLOGY

1.2.1 Lichen morphology and terminology

In 1982, the International Association of Lichenology defined lichen as an "association of a fungus and a photosynthetic symbiont resulting in a stable thallus of specific structure". Therefore, lichens are symbiotic organisms containing at least two organisms: a fungal partner, the mycobiont, and one or more photosynthetic partners, acting as photobionts, that may either be green algae or cyanobacteria. This has been such an evolutionary success that there are close to 14.000 species of lichens in the world (Brodo *et al*., 2001), whose structures have developed into a complexity that is not reached elsewhere in the fungal kingdom (Honegger, 1991).

Lichens are traditionally divided into three basic growth forms: crustose, foliose and fruticose. Crustose lichens form crusts so tightly attached to the substrate they grow on that they can't be removed without damaging the surface. Their entire lower surface grows on and among the particles of the substrate without an intervening lower cortex. Foliose lichens are somewhat leaf-like, composed of lobes with easily distinguished upper and lower surfaces. They are relatively loosely attached to their substrates, usually by means of rhizines. Fruticose lichens are usually round in cross section, and mostly branched. They can resemble little shrubs growing upward, or hang down in long strands.

Like other non-lichenized fungi, most lichens present a sexual and an asexual life cycle (Nash III, 1996). In sexual reproduction, spores are produced inside an ascus. The asci are grouped in structures called ascomata. The apothecium, the most common type of ascoma, is shaped like an open disc (Brodo et al., 2001; Purvis, 2000).

In sexual reproduction, only the fungal partner is reproduced. Therefore, the main problem of sexual reproduction within lichenization is the need for fungal spores to meet the proper photosynthetic partner in order to reestablish the symbiosis (Nash III, 1996). Many lichens increase their chances of success by reproducing vegetatively, by means of special propagules containing both fungus and photobiont. The most frequent vegetative propagules are soredia (clusters of photobiont cells enveloped by fungal hyphae), and isidia (tiny, cylindrical or granular outgrowths of the thallus that are covered with the same layer of cortex as the thallus) (Brodo et al., 2001; Purvis, 2000).

1.2.2 Lichens and the forest ecosystem

1.2.2.1 The importance of epiphytic lichen conservation

Forests are species-rich ecosystems supporting a wide array of taxa from numerous groups ranging from birds and canopy arthropods to soil microbes. The long-term maintenance of such biological diversity represents a major management challenge in forests (Lindenmayer *et al*., 2006; Pykala *et al*., 2006) for both scientists and land managers.

Epiphytic lichens play several important funcional roles in forest ecosystems. They absorb significant amounts of nutrients from rainwater that passes through the canopy and differentially absorb minerals flowing down the trunks, thereby influencing the composition and concentration of nutrients in the soil below trees. They have the ability to capture fog and dew and this is important for conserving moisture where water is scarce (Brodo *et al*., 2001). They act as carbon sinks by consuming carbon dioxide used in photosynthesis and therefore play a part in delaying global warming. Where lichens cover the ground, they prevent soils from drying out and protect against erosion. On nutrient-poor soils they accumulate and release nutrients required by forest trees for growth. Nitrogen leached from, or fixed by, lichens containing cyanobacteria is important for forest development (Purvis, 2000). Lichens increase strutural complexity, influence nutrient cycling and provide habitat, food and nest material for many animals (Galloway, 1992).

In the last 50 years, concern about the loss of lichen diversity associated with forest management and forest fragmentation has led to many studies designed to assess patterns and monitor trends of lichen biodiversity in forests worldwide (Gu *et al*., 2001). Region-wide studies have goals of monitoring the relation of major factors such as climate (Ellis *et al*., 2007), air quality (Castello & Sketr, 2005; Giordani *et al*., 2002; Mayer *et al*., 2009; Saipunkaew *et al*., 2007), forest management (Nascimbene *et al*., 2007) and forest tree species composition (Coxson & Stevenson, 2007; Hilmo & Sastad, 2001; Rogers & Ryel, 2008) to variation in lichen diversity, and how these relations interact and change over time. Studies of lichens of particular forest types often have explicit goals of monitoring effects of forest management practices and landscape context, including a variety of indirect human impact on forest environments (Wolf *et al*., 2000a).

One of the most currently important research agendas is to assess the magnitude of the impact of fire on individual lichen species and lichen community composition, especially in the Mediterranean (Longán *et al*., 1999).

1.2.2.2 Habitat characteristics and environmental variables important for epiphytic lichens

The geographic distribution of epiphytic lichens varies with most of the same large-scale environmental variables to which other biota respond. Climate and its interaction with landform explain most of the variation in lichen species composition with elevation, topography, and oceanic versus continental climate. Shade, moisture, and availability of woody substrates make forested versus non-forested habitat important (Nash III, 1996). Habitat fragmentation and other human land use variables, such as urbanization, intensity of agricultural or pastoral use, and forestry management, are increasingly important as predictors of lichen species distribution.

Most lichens have some degree of substrate specificity (Nash III, 1996), so lichen community composition varies with such substrate variables as tree bark, trunk roughness and trunk pH (Brodo *et al*., 2001; Nash III, 1996). Epiphytic lichen species composition also varies with within-site differences in light regime and moisture status, so slope and aspect of tree trunk, shading by macrovegetation, position in forest layers, gaps, or edges (Bélinchon *et al.*, 2007), tree age, and dead wood are examples of microhabitat variables of importance to lichens. Epiphytic lichen distribution and biomass is heterogeneous worldwide because of the importance of small variations in microhabitat characteristics (Wolf *et al*., 2000a).

1.2.3. Lichens in biodiversity and ecosystem monitoring

1.2.3.1. Lichens as surrogate indicators for biodiversity and ecosystem function

The concept and use of indicators, particularly indicator species, has received increasing attention for application in ecologically sustainable forest management. The concept is potentially important given the impossibility of managing the huge array of taxa that inhabits forest ecosystems (Lindenmayer *et al*., 2006). Landres *et al*. (1988) defined an indicator species as ‘‘an organism whose characteristics (e.g. presence or absence, population density, dispersion, reproductive success) are used as an index of attributes to difficult, inconvenient, or expensive to measure for other species or environmental conditions of interest.’’ The indicator species approach is widely used as a measure of ecologically sustainable forest management (Lindenmayer *et al*., 2006). Lichen communities have been demonstrated to be useful indicators for the condition of many different ecosystems. For example, Nascimbene *et al*. (2007) tested Lobaria pulmonaria as a potential indicator of sites worthy of conservation in a temperate forest in northern Italy. Stofer *et al*. (2006) related species richness of lichen functional groups with land use intensity and concluded that widespread, abundant species were more likely to occur with increasing land use intensity. Rogers & Ryel (2008) concluded that epiphytic lichens communities may constitute an effective indicator of community level diversity for aspen-dependent species, when studding lichen community change in response to succession in aspen forests of the southern Rocky Mountains. More than a decade ago, Kuusinen (1996) had shown that the occurrence of cyanolichens (lichens with cyanobacteria as the main photobiont) is a potential simple indicator of the continuity of the forest stand. Hundreds of studies have linked lichen communities to air quality (e.g., Asta *et al*., 2002; Hawksworth & Rose, 1970), and several long-term lichen monitoring programs in Europe are using lichens to assess climate change (Van Herk *et al*. 2002).

Lichens are therefore able to accomplish the criteria that define a good indicator (they are widely distributed on a global scale, form perennial bodies, live in all types of substrata, are usually long-lived and highly habitat-specificity) and may be used as indicators in several different ways. These include assessing physiological, biochemical or morphological changes (Stofer *et al*., 2006), or changes in community structures through extinction or species substitution (Rogers & Ryel, 2008). The dual nature of the lichen association and its sensitivity to environmental disturbance is a major reason why lichens are useful. If the delicate balance between symbionts is disturbed, it can lead ultimately to the death of the lichen. Not all lichens respond in an identical fashion; different species show varying levels of sensitivity to particular environmental factors or changes (Purvis, 2000).

1.2.3.2. Using subsets of lichen species for monitoring

In many parts of the world, there are not enough trained technicians to do all the monitoring that is needed, nor is there enough money to pay for expert personnel to do the monitoring, so protocols which can be performed by nonspecialists are the best choice for such areas. This usually means that monitoring is restricted to a few, easily recognized, species subsets proved to act as surrogates of overall species diversity.

The two most commonly selected kinds of subsets of lichen species for monitoring are habitat subsets and lichen functional groups. Commonly used habitat subsets include epiphytic lichens on tree trunks. Although data quality is equally high for all substrates and epiphytic lichens only (Wolf *et al*., 2000b), the advantage of restricting a survey to epiphytic lichens is the considerable time and cost reduction.

The term functional group is used in ecology to refer to a group of organisms whose functional roles in the community are similar (Wolf *et al*., 2000a). In the particular case of lichens, functional groups are usually defined based on morphological similarity and presumed functional similarity, as it happens when using cyanolichens as a functional group. Some species appear to be more tolerant than others to ecosystem changes and this is probably due to differences in their morphologies. Therefore functional and morphological groups of lichens can provide valuable insights into the ecosystem heath and recovery. When macrolichens are sufficiently common, they are frequently monitored as indicators of response to all lichens (Bergamini *et al.*, 2005, 2007) since the identification of most crustose species requires expert knowledge. Dividing species into functional groups after field registry to aid in data interpretation and prediction is more common than recording data in the field by such groups since it has been recognized that functional groups are more valuable as indicators of ecosystem function rather than for biodiversity monitoring purposes (Wolf *et al.*, 2000b).

1.2.4. Epiphytic lichens and forest fires

1.2.4.1. Forest fire ecology

Fire is a natural disturbance and a forest management technique. It is part of the forests ecosystem dynamics as well as the evolutionary environment (Goldammer & Furyaev, 1996). According to intensity, frequency, and physical characteristics of the landscape or ecosystem, fire is a factor that shapes species composition, age, and canopy structure of many ecosystems (Norton & Lange, 2003; Pyne *et al.*, 1996). Fire can also improve the regeneration potential of tree forests (e.g. oaks) by: (1) reducing tree density and canopy cover thus increasing light levels on the forest floor, (2) reducing competition from more fire sensitive saplings and seedlings in the understory, and (3) creating seedbed conditions more favorable to establishment (Hutchinson *et al.*, 2005). In the past, forests were burned and converted into agricultural fields, today fire is still used in traditional land use systems, such as agriculture and silviculture (Goldammer & Furyaev, 1996). Consequently, the natural fire regimes have been severely altered (Goldammer, 2003, 2004). After a forest fire, a secondary succession begins. Species succession is a temporal sequence of appearance and disappearance of species. The presence of species depends on interactions between biotic and abiotic processes, which influence different stages of a species' life history over time (Holmes, 2003). The potential force of a fire turns it into a complex phenomenon, which, if employed without adequate knowledge, can threaten the biological productivity, biodiversity, and sustainability of ecosystems (World Conservation Union, 2003).

1.2.4.2. Fire disturbance in lichen communities

In 1994, Huston defined disturbance as "any process or condition external to the natural physiology of living organisms that results in the sudden mortality of biomass in a community on a time scale significantly shorter than that of the accumulation of the biomass".

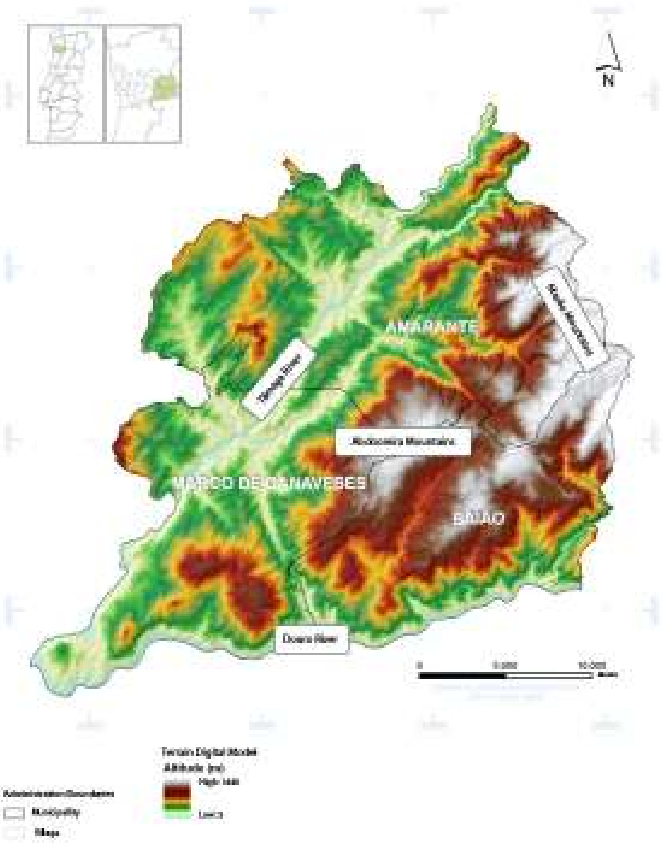
Lichens are poikilohydric organisms, which lack physical structures such as a cuticle and stomata to regulate gas exchange. As a consequence, they require some adaptive protection to environmental conditions. The fungal component is important for physical protection of the algal layer against such things as pollution, high levels of light (Nash III, 1996), and, possibly, fire. Additionally, lichens are only metabolically active when wet. Therefore, the fact that lichens are probably dry and metabolically inactive when partially burned may greatly affect their ability to regenerate from fragments. Compared with other organisms, such as vascular plants, lichens are characterized by slow growth rates, therefore, disturbance, such as fire, should have long-lasting effects on the lichen community (Atlas *et al.*, 2004; Johansson *et al.,* 2005). These effects also seem to agree with the often-observed high abundance and diversity of lichens in "old-growth" forest, in contrast to younger forest stages (Hilmo & Sastad, 2001).

A fairly extensive line of research on disturbance effects on lichens is concerned with the consequences of land use, mainly forestry (Stofer *et al.*, 2006). These studies include, for example, comparisons of lichen diversity between old growth and young forests (Kuusinen & Siitonen, 1998), and of lichen response to forest edges (Belinchón *et al.*, 2007). In contrast to the numerous studies on the impact of forestry and forest age on epiphytic lichens, there are, however, surprisingly few studies on epiphytic lichens and natural disturbance, such as wildfire (Longán *et al.,* 1999).

Thus, it is evident that there is a gap regarding our understanding of the role of natural disturbances for epiphytic lichens in forest ecosystems. Filling this gap is especially urgent in a conservation biology context, where the importance of "old-growth" forest is often stressed and used as reference when evaluating biodiversity in managed landscapes (Hilmo & Sastad, 2001). So one of the most crucial questions is how lichens recolonize regenerating forests following natural disturbance (Johansson, 2008). This knowledge is important to evaluate the potential to restore and maintain forest biodiversity by using management models based on natural disturbance regimes and their variability (Lindenmayer *et al*., 2006; Niemela, 1999).

1.3. STUDY AREA

1.3.1. Location and general ecology



Aboboreira and Castelo are a mountain area located in north-western Portugal (at the junction between the municipalities of Amarante, Baião, and Marco de Canaveses) with a total area of *ca.* 105 km^2 and reaching approximately 1000m a.s.l, with a NE-SO orientation and surrounded by the valleys of the Ovelha, Ovil, Fornelo and Douro rivers (figure 1). Geologically this mountain belongs to the Center-Iberian Zone as defined by Ribeiro *et al.* (1979), which is formed essentially by granitic and metasedimentary rocks (Honrado & Vieira, 2009).

Figure 1. Location and geographic context of the study area. (Modified from Honrado *et al.*, 2009).

The climate in Aboboreira and Castelo mountain can be considered Oceanic, which translates the Atlantic Ocean influences on temperatures, frequent fogs, abundant precipitation (AP: 1500-2000 mm) and an annual average temperature of 13ºC. With increasing elevation, temperature decreases and precipitation increases. Yearly insolation varies between 2300 and 2700 h/year and solar radiation varies between 140 and 150 kcal/cm^2. The number of days per year with precipitation diverges from 75 to 100 days and the air humidity rounds 75% (Instituto do Ambiente, 2008). However, summers are relatively dry, reflecting a (sub)Mediterranean influence, characteristic of the Iberian climate (Honrado & Vieira, 2009).

The typical forest vegetation of these mountains consists of oak woods dominated by *Quercus robur*. This type of ecosystem hosts many forest plant species like *Ilex aquifolium*, *Ruscus aculeatus, Saxifraga spathularis* and the endemic *Anemone trifolia* subsp. *albida*. In dry and sunny slopes we can find *Quercus suber* and *Quercus pyrenaica*. Phytosociologically these oak woods are classified into the *Rusco aculeati-Quercetum roboris* association. The degradation of *Quercus* forest has lead to the expansion of herbaceous vegetation and scrublands dominated by *Cytisus striatus*.

During the last two decades, other considerable modifications in land use took place. The observed increase of total uncultivated land was partly related to the abandonment of agriculture but mainly to the loss of forest patrimony by logging and fire. Forest plantations of fast-growing tree species like *Pinus pinaster* and *Eucalyptus globulus* have become an increasingly important land use in Aboboreira and Castelo, where native oak woodland have been converted to such plantations (Honrado & Vieira, 2009).

The importance of the natural patrimony still occurring in these mountains, together with their well-known archaeological richness, has lead to innumerous initiatives concerning his protection. The most important one was the political proposal to classify the Aboboreira and Castelo mountains as "Área de Paisagem Protegida" (Projecto de Lei n.º 138/VIII), back in year 2000.

1.3.2. Wildfires in the Baixo Tâmega

Forest fire is one of the worst natural catastrophes in Baixo Tâmega, geographic region where Aboboreira and Castelo mountain are located, not only because of their high frequency and wide extension but also because of their enormous destructive effects. Fires lead to financial loss and to the end of goods and services provided by the forests. Historically, this region presents a vast series of fire occurrences, most of them in mountain areas with forest or grazing land use.

According to the National Forest Authority (2008) report between 1990 and 2007 a total of 748 occurrences consumed more than 41000 ha of forest and scrublands in Baixo Tâmega (figure 2). The most problematic years were 1998 and 2005, with 10188 e 8382 ha of burned area, respectively. The slopes of the Aboboreira and Castelo mountains, where our surveys were conducted, were severely and recurrently affected by wildfires in this period (figure 2), thus providing a suitable fire disturbance gradient for ecosystem resilience studies.

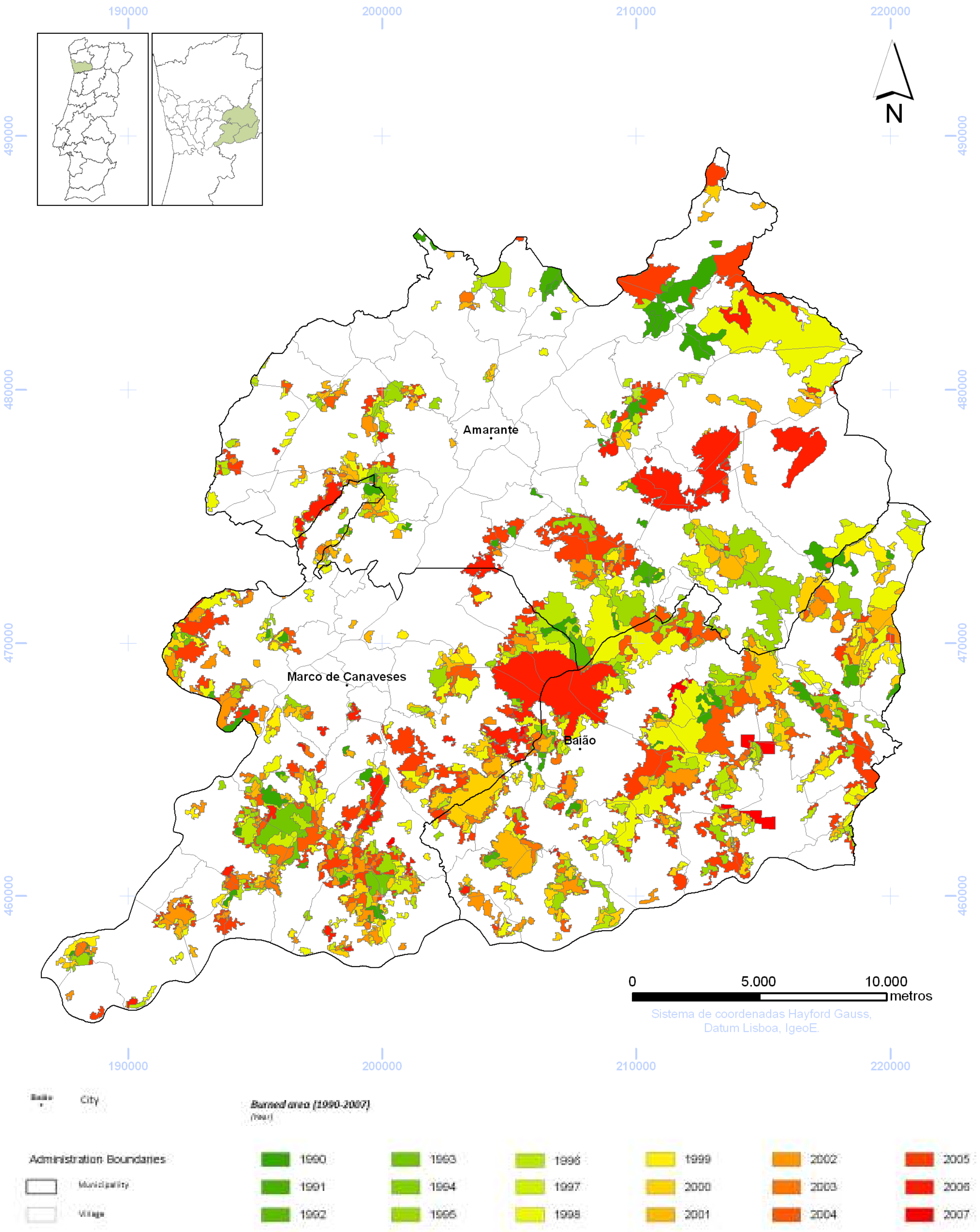


Figure 2. Burned areas in the Baixo Tâmega region between 1990 and 2007. (Modified from Honrado & Vieira, 2009)

1.4. DETAILED METHODOLOGY FOR THE TAXONOMIC DETERMINATION OF COLLECTED LICHEN SPECIMENS

1.4.1. **Collecting and processing of specimens**

Specimen collection was carried out with the aid of a pocket knife. The collected specimens were preserved in paper bags properly identified with respective date and inventory code, and transported to the laboratory for separation, drying, and identification. Excess substrate was carefully removed from the gathered specimens.

Separation of all specimens was based on the observation of the most evident morphological differences. Once isolated, the specimens were placed in individual envelopes which include information on Date and Place of collection (Town, County, and Geographical Position), Collector, Phenology, Chemical Reactions of thallus and medulla among others.

1.4.2. Taxonomic determination of collected specimens

1.4.2.1. *Morphology and Anatomy*

The identification was based on the dichotomous keys from general floras, including the European flora from Clauzade & Roux (1985) and the flora of the United Kingdom of Purvis *et al.* (1992). When available, monographs of specific genera were used for identification to the species level, including Boqueras *et al.* (1999, 2003) for the genera *Pertusaria* and *Ochrolechia,* Burgaz (2007) for the genus *Cladonia,* Fos & Clerc (2000) for the genus *Usnea* and Giralt *et al.* (2000) for genus *Buellia.* The names of the remaining species were obtained using dichotomous keys of the already mentioned European floras and later updated accordingly to Hladun (2000-2007).

In the process of identification a stereomicroscope was used to observe numerous morphological features such as shape, color and size of the thallus; presence of vegetative propagules and their form and distribution in the thallus; presence and type of rizines; presence of cifel or pseudocifels, among others.

The anatomical features of thallus and their reproductive structures were observed under an optical microscope with objective lenses up to 100x, including the characteristics of apothecia, shape, color and size of spores, and the number of spores per ascus. All cuts were performed "freehand" with a razor blade. All measurements were made in an aqueous solution using a micrometer eyepiece.

1.4.2.2. *Chemotaxonomy*

To assist the identification process, several commonly used chemical reagents in lichen chemotaxonomy were applied, namely, solution of potassium hydroxide 10% (K test), solution of sodium hypochlorite or commercial bleach (C test) and alcohol solution of *para*-phenylenediamine (P test). These reagents were applied with the aid of a capillary in the bone marrow and/or cortex, as indicated in the keys used. Medullary tests were performed by removing a small fraction of the cortex with a razor blade.

The color change resulting from application of these reagents is indicative of the presence of lichen substances with taxonomic significance and has been duly noted. All positive reactions were indicated by a plus sign (+) followed by an indication of the resulting color; negative reactions were indicated by a minus sign (-). It was given particular attention to the fact that some reactions only revealed a color change after a short amount of time, so, only after a few moments was the result of the reaction registered.

Chemical tests were conducted in parts of the young thallus and carried out under the stereomicroscope with white light where color changes like yellow were not considered false negatives.

Whenever any specimen presented difficulties for identification using only the morphological and anatomical features, thin layer chromatography was used according to the methodology proposed by Orange *et al.* (2001) for identification of chemical compounds of taxonomic significance. The extracts of the specimens to identify were obtained with acetone, and placed in a 20x20 cm aluminum plates coated with silica gel. Two patterns were used, atranorina and norstictic acid. These are present in *Platismatia glauca* and *Pleurosticti acetabulum* extracts, respectively, which were used as control.

A parallel line to the plate base was drawn at 1 cm, and on it were placed the lichen extracts with a spacing of 1 cm between them using a glass capillary. The placement of the extract was performed repeatedly until the entire solution was used. Each sample was assigned a number registered in its herbarium envelope. Another line was drawn on the board at 2 cm from the top to mark the end of the run of the solvent.

After putting the extracts, the plates were placed in two chromatographic tins with their respective solvent: solvent A (toluene / 1,4-dioxane / acetic acid, 180:45:5) and solvent C (toluene acid / acetic acid, 170:30). To facilitate the rise of solvent C, the plates was first placed for 10 minutes in a tank with air saturated with acetic acid. After migration of the solvent, the plates were sprayed with 10% sulfuric acid and placed in an oven for about 10 minutes at 110 ^{o}C. After heating up, the plates revealed a range of smudges whose colors and itinerary across the plate are characteristic of each compound.

Among the specimens subjected to thin layer chromatography were the ones belonging to the genera *Cladonia* and *Usnea.* The interpretation of data from chromatography was based on tables proposed by Orange *et al.* (2001).

2. INTRODUÇÃO

2.1 OBJECTIVOS E ORGANIZAÇÃO GERAL DA TESE

Este estudo foi levado a cabo no âmbito de um projecto mais abrangente com vista à promoção do património natural e cultural enquanto factores de desenvolvimento e competitividade territoriais na região do Baixo Tâmega (Norte de Portugal). Os principais objectivos do projecto são a caracterização e valoração do património natural deste território e a determinação da sua vulnerabilidade a diversos factores de mudança (Honrado *et al.,* 2009).

A Serra de Aboboreira e Castelo, localizada no coração do Baixo Tâmega, é conhecida pela prevalente regeneração de jovens carvalhais resultante do abandono agrícola e da pastorícia. No final do Verão de 2009, ocorreram incêndios de grandes proporções na região, revelando a importância do fogo como um factor de gestão da vegetação e da biodiversidade. Neste contexto, este estudo apresenta três grandes objectivos: (1) determinar a diversidade dos líquenes epífitios em carvalhais da Serra da Aboboreira e Castelo, (2) examinar o impacto que o fogo teve sobre os líquenes epífitos e os padrões de recuperação da comunidade epifítica, e (3) avaliar o uso potencial de espécies ou grupos liquénicos seleccionados como indicadores de biodiversidade, num cenário pós-fogo.

Esta tese inclui resultados de três diferentes estudos conduzidos na Serra da Aboboreira e Castelo. Assim, este estudo foi efectuado para determinar a riqueza específica de líquenes epifíticos, os seus padrões de distribuição e a sua resposta à perturbação ao longo de um gradiente pós-fogo.

De acordo com os objectivos, esta tese foi organizada em três estudos, um por cada objectivo geral.

2.2 BREVE ABORDAGEM À ECOLOGIA DE LÍQUENES EPÍFITOS

1.2.1 Morfologia e terminologia liquénica

Em 1982, a Associação Internacional de Liquenologia definiu os líquenes como uma "associação entre um fungo e um simbionte fotossintético de que resulta um talo estável com uma estrutura específica". Portanto, os líquenes são organismos simbióticos contendo no mínimo dois organismos: um parceiro fúngico, o micobionte, e um ou mais parceiros fotossintéticos, actuando como fotobionte, que poderão ser algas verdes ou cianobactérias. Isto representa um enorme sucesso evolutivo e estima-se que existam aproximadamente 14.000 espécies liquénicas no Mundo (Brodo *et* *al*., 2001), que atingiram uma enorme complexidade estrutural sem comparação no Reino Fungi (Honegger, 1991).

Os líquenes são tradicionalmente divididos em três formas básicas de crescimento: crustáceos, foliáceos e fruticolosos. Os líquenes crustáceos formam crostas de tal forma presas ao substrato onde crescem que não é possível removê-los sem que se danifique a superfície. Toda a sua superfície inferior cresce sobre e entre as partículas do substrato sem intervenção de um córtex inferior. Os líquenes foliáceos parecem-se com folhas, possuem lóbulos onde facilmente se distingue uma superfície superior e uma superfície inferior. Possuem fraca adesão ao substrato, usualmente efectuada através de rizinas. Usualmente em corte transversal os líquenes fruticolosos são redondos e são na sua maioria ramificados. Podem assemelhar-se a pequenos arbustos ou a longos filamentos.

Tal como outros fungos não liquenizados, a maioria dos líquenes apresenta ciclos de reprodução sexuada e assexuada (Nash III, 1996). Na reprodução sexuada cada um dos componentes reproduz-se por si mesmo, no entanto, têm de se reencontrar para que seja possível recriar o líquen. Assim, a maior dificuldade da reprodução sexuada na liquenização é a necessidade dos propágulos do fungo encontrarem o parceiro fotossintético apropriado de modo a restabelecer a simbiose (Nash III, 1996). Muitos líquenes aumentam as suas probabilidades de sucesso através da reprodução vegetativa (assexuada), por meio de propágulos especiais que contêm tanto o fungo como o fotobionte. Os propágulos vegetativos mais frequentes são os sorédios (agregados de células do fotobionte rodeadas por hifas do fungo) e os isídios (projecções do talo que podem ser pequenas, cilíndricas ou granulares, sendo cobertas pela mesma camada de córtex que o talo) (Brodo *et al*., 2001; Purvis, 2000).

1.2.2 Líquenes e ecossistemas florestais

1.2.2.1 A importância da conservação dos líquenes epifíticos

As florestas são ecossistemas ricos em espécies e suportam uma grande variedade de *taxa* de numerosos grupos desde pássaros e artrópodes da canópia até micróbios do solo. A manutenção desta diversidade biológica representa um enorme desafio para a gestão dos ecossistemas florestais (Lindenmayer *et al*., 2006; Pykala *et al.*, 2006) tanto para os cientistas como para os gestores do território. Os líquenes epífitos desempenham importantes funções nos ecossistemas florestais. Eles absorvem quantidades significativas de nutrientes provenientes das chuvas que atravessam a canopia e absorvem de forma diferencial os nutrientes que descem pelo tronco, influenciando a composição e a concentração de nutrientes no solo. Possuem a capacidade de capturar o nevoeiro e a neblina o que se revela de máxima importância na conservação da humidade em locais onde a água é escassa (Brodo *et al*., 2001). Actuam como reservatório de carbono através do consumo do dióxido de carbono usado na fotossíntese e como tal desempenham um importante papel no retardar do aquecimento global. Nos locais onde os líquenes cobrem o solo, previnem a erosão e conservam a humidade. Nos solos pobres em nutrientes acumulam e libertam os nutrientes requeridos para o crescimento das espécies vegetais. O azoto libertado ou fixado pelos líquenes que contêm cianobactérias é importante para o desenvolvimento florestal (Purvis, 2000). Os líquenes aumentam a complexidade estrutural, influenciam o ciclo dos nutrientes e providenciam habitat, alimento e material para a construção dos ninhos (Galloway, 1992).

Nos últimos 50 anos preocupações relativas à perda de diversidade liquénica associada à gestão e fragmentação florestal levou ao desenvolvimento de muitos estudos com objectivo de determinar os padrões e monitorizar tendências da diversidade liquénica nas florestas de todo o mundo (Gu *et al*., 2001). Estes estudos têm como objectivos a monitorização da relação entre factores como o clima (Ellis *et al*., 2007), qualidade do ar (Castello & Sketr, 2005; Giordani *et al*., 2002; Mayer *et al*., 2009; Saipunkaew *et al*., 2007), gestão florestal (Nascimbene *et al*., 2007) e composição das espécies arbóreas (Coxson & Stevenson, 2007; Hilmo & Sastad, 2001; Rogers & Ryel, 2008) com a variação da diversidade liquénica, e como essa relação varia ao longo do tempo. Estudos que visam os líquenes de um tipo particular de floresta muitas vezes têm como objectivo a monitorização dos efeitos provocados pelas práticas de gestão florestal e o contexto da paisagem, incluindo diversos factores de impacto humano indirecto nos ambientes florestais (Wolf *et al*., 2000a).

Actualmente, uma das linhas de pesquisa mais importante é a determinação da magnitude do impacto do fogo nas espécies e na composição da comunidade liquénica, especialmente na região Mediterrânica (Longán *et al*., 1999).

1.2.2.2 Características de habitat e variáveis ambientais importantes para os líquenes epífitos

A distribuição geográfica dos líquenes epífitos varia na mesma escala que as variáveis ambientais a que outros biota respondem. O clima e a sua interacção com a morfologia explicam a maioria das variações na composição das espécies liquénicas, tais como a elevação, a topografia, e o clima oceânico versus o continental. A sombra, a humidade e a disponibilidade de madeira para substrato são de extrema importância quando consideradas as diferenças entre habitats florestais e não florestais (Nash III, 1996). A fragmentação dos habitats e outras alterações humanas do uso do solo, tais como, a urbanização, a intensidade agrícola ou pastorícia, a gestão florestal, são cada vez mais importantes enquanto previsores dos padrões de distribuição liquénica.

Muitos líquenes possuem especificidade de substrato (Nash III, 1996), assim, a comunidade liquénica varia com a variação de substrato, incluindo o tipo de casca , rugosidade e pH do tronco (Brodo *et al*., 2001; Nash III, 1996). A composição dos das comunidades epifíticas também varia conforme as variações de luz e condições de humidade, inclinação e aspecto do tronco, sombra causada pela macrovegetação, localização na floresta, espaço entre árvores, idade do substrato arbóreo e existência de orlas e árvores mortas(Bélinchon *et al.*, 2007). A distribuição da biomassa liquénica epifítica no Mundo é heterogénea e resulta de pequenas variações nas características dos microhabitats (Wolf *et al*., 2000a).

1.2.3. Líquenes na monitorização da biodiversidade dos ecossistemas

1.2.3.1. Líquenes como indicadores de biodiversidade e do funcionamento do ecossistema

A concepção e o uso de indicadores, particularmente espécies indicadoras, tem recebido atenção crescente para aplicação em gestão florestal ecologicamente sustentada, o conceito é potencialmente importante dada a impossibilidade de gerir o gigantesco número de *taxa* que habita um ecossistema florestal (Lindenmayer *et al.,* 2006). Landres *et al.* (1988) definiu espécie indicadora como "um organismo cujas características (ex. presença ou ausência, densidade populacional, dispersão, sucesso reprodutor) são usadas como um catálogo de atributos demasiado difícil, inconveniente ou caro de medir para outras espécies ou condições ambientais de interesse." A directiva de espécies indicadoras é universalmente usada como uma medida de gestão florestal com sustentabilidade ecológica (Lindenmayer *et al.*, 2006). A comunidade liquénica tem demonstrado ser um indicador útil para as condições de muitos ecossistemas diferentes. Por exemplo, Nascimbene *et al.* (2007) testou a *Lobaria pulmonaria* como um indicador potencial de sítios dignos de conservação em florestas temperadas do Norte de Itália. Stofer *et al.* (2006) relacionou a riqueza específica de grupos funcionais liquénicos com diferentes intensidades do uso do solo e concluiu que espécies mais abundantes e com melhor distribuição têm maior probabilidade de ocorrer em locais com maior intensidades de uso do solo. Rogers & Ryel (2008) concluíram que as comunidades de líquenes epífitos constituem um indicador eficiente de diferentes níveis de diversidade da comunidade de faias, quando estudada a variação da comunidade liquénica em resposta à sucessão de florestas de faias no sul de Rocky Mountains. Há mais de uma década, Kuusinen (1996) mostrou que a ocorrência de cianoliquenes (líquenes com cianobactérias como principal fotobionte) é um indicador potencial da situação de continuidade florestal.

Os líquenes satisfazem os critérios que definem um bom indicador (possuem ampla distribuição a uma escala global, formam estruturas perenes, vivem em todos os tipos de substrato, têm longa longevidade e especificidade de habitat) e podem ser usados como indicadores de diversas formas, incluindo a determinação de mudanças fisiológicas, bioquímicas ou morfológicas (Stofer *et al*., 2006), ou mudanças estruturais na comunidade quer através de extinção ou de substituição de espécies (Rogers & Ryel, 2008). A natureza dual da associação liquénica e a sua sensibilidade a perturbações ambientais é a principal razão da utilidade dos líquenes. Se o delicado equilíbrio entre simbiontes é perturbado pode levar, em última analise, à morte do líquen. Nem todos os líquenes respondem de forma semelhante; diferentes espécies apresentam diferentes níveis de sensibilidade a diferentes factores ou mudanças ambientais (Purvis, 2000).

1.2.3.2. O uso de grupos funcionais liquénicos em monitorização

Em muitas partes do mundo, não existem técnicos treinados suficientes para executar toda a monitorização necessária, nem existem fundos suficientes para pagar a especialistas para fazer a monitorização, assim protocolos passíveis de serem executados por não especialistas são a melhor opção para essas áreas. Isto normalmente significa que a monitorização será restrita a algumas, espécies, facilmente reconhecíveis, que tenham demonstrado ser indicadoras da diversidade geral.

As duas divisões de espécies liquénicas mais comuns na monitorização são a divisão por habitats e por grupos funcionais liquénicos. Comummente as divisões por habitat referem-se aos líquenes existentes nos troncos de árvores. No entanto, a qualidade dos dados é igualmente elevada para os restantes substratos (Wolf *et al*., 2000b). A vantagem de restringir o estudo aos líquenes epífitos é a grande redução de custo e tempo. O termo grupo funcional é usado em ecologia para se referir a um grupo de organismos que desempenham as mesmas funções ecológicas na comunidade (Wolf *et al*., 2000a). No caso particular dos líquenes, os grupos funcionais são usualmente definidos com base em similaridades morfológicas e presumidas semelhanças funcionais, como acontece com os cianolíquenes. Algumas espécies parecem ser mais tolerantes a mudanças nos ecossistemas e isso deve-se muito provavelmente a diferenças morfológicas. Assim, grupos funcionais e morfológicos de líquenes podem fornecer dados importantes sobre a saúde dos ecossistemas e a sua recuperação. Quando os macrolíquenes são suficientemente comuns são frequentemente monitorizados como indicadores da resposta de todos os líquenes (Bergamini *et al.*, 2005, 2007) uma vez que a identificação da maioria dos crustáceos requer conhecimento especializado.

1.2.4. Líquenes epifiticos e fogos florestais

1.2.4.1. Ecologia do fogo florestal

O fogo é uma perturbação natural e uma forma de gestão florestal. Faz parte da dinâmica dos ecossistemas assim como da evolução ambiental (Goldammer & Furyaev, 1996). De acordo com a sua intensidade, frequência e características físicas da paisagem ou do ecossistema, o fogo é um factor que molda a composição específica, a idade e a estrutura da canópia de muitos ecossistemas (Norton & Lange, 2003; Pyne *et al*., 1996). O fogo também melhora a regeneração potencial das árvores florestais (ex. carvalhos) através: (1) redução da densidade e cobertura da canópia, o que permite maior intensidade de luz no coberto florestal, (2) reduz a competição de espécies e sementes mais sensíveis ao fogo, e (3) cria condições mais favoráveis ao estabelecimento das sementes (Hutchinson *et al.*, 2005). No passado, as florestas eram queimadas e transformadas em campos agrícolas, nos dias de hoje o fogo é ainda usado nos métodos tradicionais de uso do solo, tais como agricultura e silvicultura (Goldammer & Furyaev, 1996). Consequentemente, o regime natural do fogo foi severamente alterado (Goldammer, 2003, 2004). Após um fogo florestal inicia-se uma sucessão secundária. A sucessão de espécies é uma sequência temporal de aparecimento e desaparecimento de espécies. A presença de espécies depende da interacção entre os processos bióticos e abióticos, que influenciam diferentes estádios da história de vida das espécies ao longo do tempo (Holmes, 2003). As características do fogo tornam-no num fenómeno complexo, que se usado sem conhecimento adequado, pode ameaçar a produtividade biológica, a biodiversidade e a sustentabilidade dos ecossistemas (World Conservation Union, 2003).

1.2.4.2. Perturbação pelo fogo nas comunidades liquénicas

Em 1994, Huston definiu perturbação como "um processo ou condição externa à fisiologia natural do organismo vivo que resulta numa mortalidade súbdita da biomassa numa comunidade ou num período de tempo significativamente mais curto que o necessário para ocorrer acumulação de biomassa".

Os líquenes são organismos poiquilohidricos, aos quais faltam estruturas semelhantes à cutícula e aos estomas para regular as trocas gasosas. Como consequência eles necessitam adaptações que os protejam das condições ambientais. O componente fúngico é importante para a protecção física da camada algal para fenómenos como poluição, elevados índices de luz (Nash III, 1996) e possivelmente fogo. Adicionalmente, os líquenes só são metabolicamente activos quando húmidos. Portanto, o facto de os líquenes se encontrarem secos e metabolicamente inactivos quando parcialmente queimados afecta grandemente o seu poder de regeneração a partir dos fragmentos. Quando comparados com outros organismos tais como plantas vasculares, os líquenes são caracterizados por um crescimento lento, assim, perturbações, tais como fogo, possuem efeitos duradouros na comunidade liquénica (Atlas *et al*., 2004; Johansson *et al.,* 2005). Estes efeitos também estão de acordo com a grande abundância e diversidade observada nas florestas maduras em contraste com as florestas jovens (Hilmo *&* Sastad, 2001).

Uma linha de pesquisa frequente diz respeito à avaliação que os diferentes usos do solo têm sobre a comunidade liquénica e as suas consequências (Stofer *et al.*, 2006). Estes estudos incluem, por exemplo, comparações entre a diversidade liquénica existente em florestas maduras e recentes (Kuusinen & Siitonen, 1998) e a resposta liquénica ao efeito de ecótono (Belinchón *et al.*, 2007). Contrariamente ao grande número de estudos sobre o impacto das actividades florestais e da idade da floresta nos líquenes epífitos, existem surpreendentemente poucos estudos sobre o impacto que as perturbações naturais, tais como o fogo, têm sobre estes organismos (Longán *et al.,* 1999).

Existe uma lacuna evidente no que concerne o nosso conhecimento sobre a resposta dos líquenes epífitos às perturbações naturais em ecossistemas naturais. Colmatar esta lacuna é especialmente urgente no contexto biológico onde a importância das florestas prestinas é cada vez mais premente e quando são recorrentemente utilizadas como referência em avaliações da biodiversidade em paisagens alteradas (Hilmo & Sastad, 2001). Portanto, uma das questões cruciais é como se dá a recolonização liquénica em florestas em regeneração após perturbações naturais (Johansson, 2008). Este conhecimento é importante para avaliar o potencial para restaurar e manter a biodiversidade florestal através do uso de modelos de gestão baseados em regimes de perturbação naturais e na sua variabilidade (Lindenmayer *et al*., 2006; Niemela, 1999).

1.3. AREA DE ESTUDO

1.3.1. Localização e ecologia geral

A Serra da Aboboreira e Castelo é uma área montanhosa localizada no noroeste de Portugal (na junção entre os Municípios de Amarante, Baião e Marco de Canaveses) com uma área total de 105 km^2, atinge aproximadamente os 1000m, está orientada a NE-SO e é contornada pelos vales dos rios Ovelha, Ovil, Fornelo e Douro (figura 1). Geologicamente pertence à Zona Centro-Ibérica como determinado por Ribeiro *et al.* (1979), é constituída essencialmente por granito e rochas metassedimentares (Honrado & Vieira, 2009).

O clima da Serra da Aboboreira e Castelo pode ser considerado Oceânico, o que traduz a influência do Oceano Atlântico na temperatura, revelado através dos frequentes nevoeiros, das precipitações abundantes (1500-2000 mm) e de uma temperatura média anual de 13ºC. Com a crescente elevação ocorre uma diminuição de temperatura e um aumento de precipitação. A insolação anual varia entre 2300 e 2700 h/ano e a radiação solar varia entre 140 e 150 kcal/cm^2. O número de dias por ano com precipitação diverge entre 75 e 100 dias e a humidade do ar ronda os 75% (Instituto do Ambiente, 2008). Contudo o Verão é relativamente seco, reflectindo uma influência (sub)Mediterrânica, característica do clima Ibérico (Honrado & Vieira, 2009).

A vegetação florestal típica destas montanhas consiste em florestas de carvalhos dominadas por *Quercus robur*. Este tipo de ecossistemas alberga muitas espécies de plantas florestais como a *Ilex aquifolium*, *Ruscus aculeatus, Saxifraga spathularis* e a endémica *Anemone trifolia* subsp. *albida*. Nas encostas secas e solarengas é possível encontrar *Quercus suber* e *Quercus pyrenaica*. Fitossosiologicamente estas florestas de carvalhos estão classificadas na associação *Rusco aculeati-Quercetum roboris*. A degradação das florestas de *Quercus* levou a uma grande expansão de vegetação herbácea dominada por *Cytisus striatus*. Durante as últimas duas décadas, outras importantes modificações no uso da terra tiveram lugar. O observável aumento de áreas não cultivadas deveu-se em parte ao abandono da agricultura, no entanto a maior parte da perda do património florestal resultou do abate de árvores e de incêndios. Florestas de plantações de espécies de árvores de rápido crescimento como *Pinus pinaster* e *Eucalyptus globulus* têm tido uma importância crescente nos usos do solo da Aboboreira e Castelo, onde os carvalhais têm sido convertidos em plantações desse tipo (Honrado & Vieira, 2009).

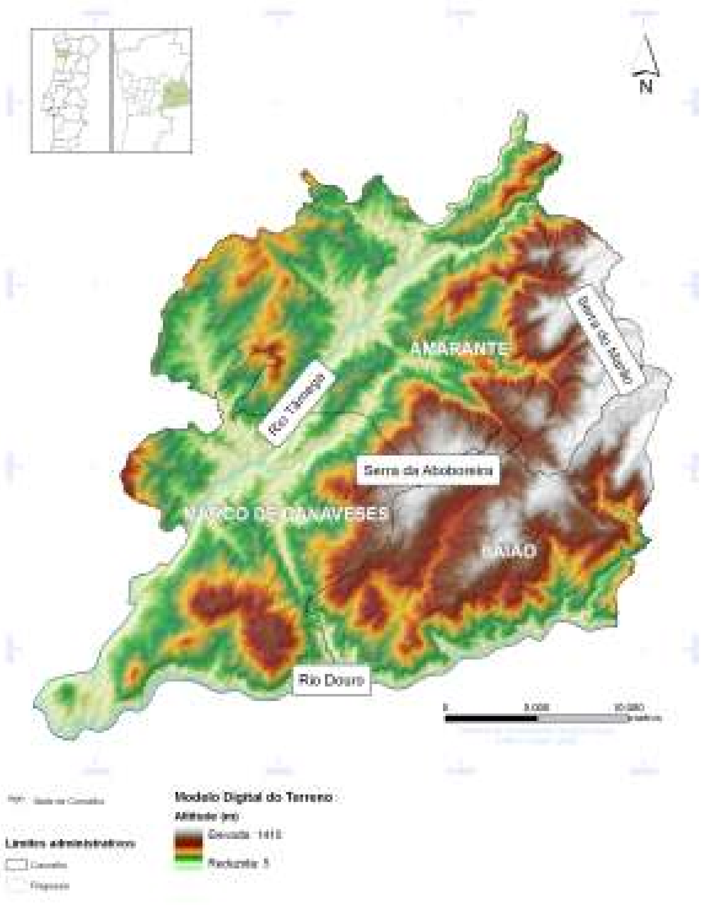


Figura 1. Localização e contexto geográfico da área de estudo.
(Retirado de Honrado *et al.*, 2009).

A importância do património natural que ainda ocorre nestas montanhas, conjuntamente com o seu riquíssimo património arqueológico, têm levado a inúmeras iniciativas para promoção da sua conservação. A mais importante diz respeito à proposta política para classificar a Serra da Aboboreira e Castelo como "Área de Paisagem Protegida" (Projecto de Lei n.º 138/VIII), no ano 2000.

1.3.2. Fogos no Baixo Tâmega

O fogo florestal é uma das principais catástrofes na região do Baixo Tâmega, região geográfica onde se localiza a Serra de Aboboreira e Castelo, não só devido à sua frequência e grande extensão, mas também devido ao seu enorme poder destrutivo. Os fogos levam a perdas financeiras e à destruição de bens e serviços fornecidos pelas florestas. Historicamente, esta região apresenta uma vasta serie de ocorrências de fogo, a maioria nas áreas montanhosas com floresta ou pastos.

De acordo com a Autoridade Nacional das Florestas (2008) foram reportados entre 1990 e 2007 um total de 748 ocorrências e foram consumidos mais de 41.000 ha de floresta na região do Baixo Tâmega (figura 2). Os anos mais problemáticos foram os de 1998 e 2005, com 10.188 e 8.382 ha de área ardida, respectivamente. As encostas da Serra da Aboboreira e Castelo, onde o nosso estudo foi conduzido, foram severa e recorrentemente afectadas pelo fogo durante esse período de tempo (figura 2), fornecendo um gradiente de fogo para estudos de recuperação dos ecossistemas.

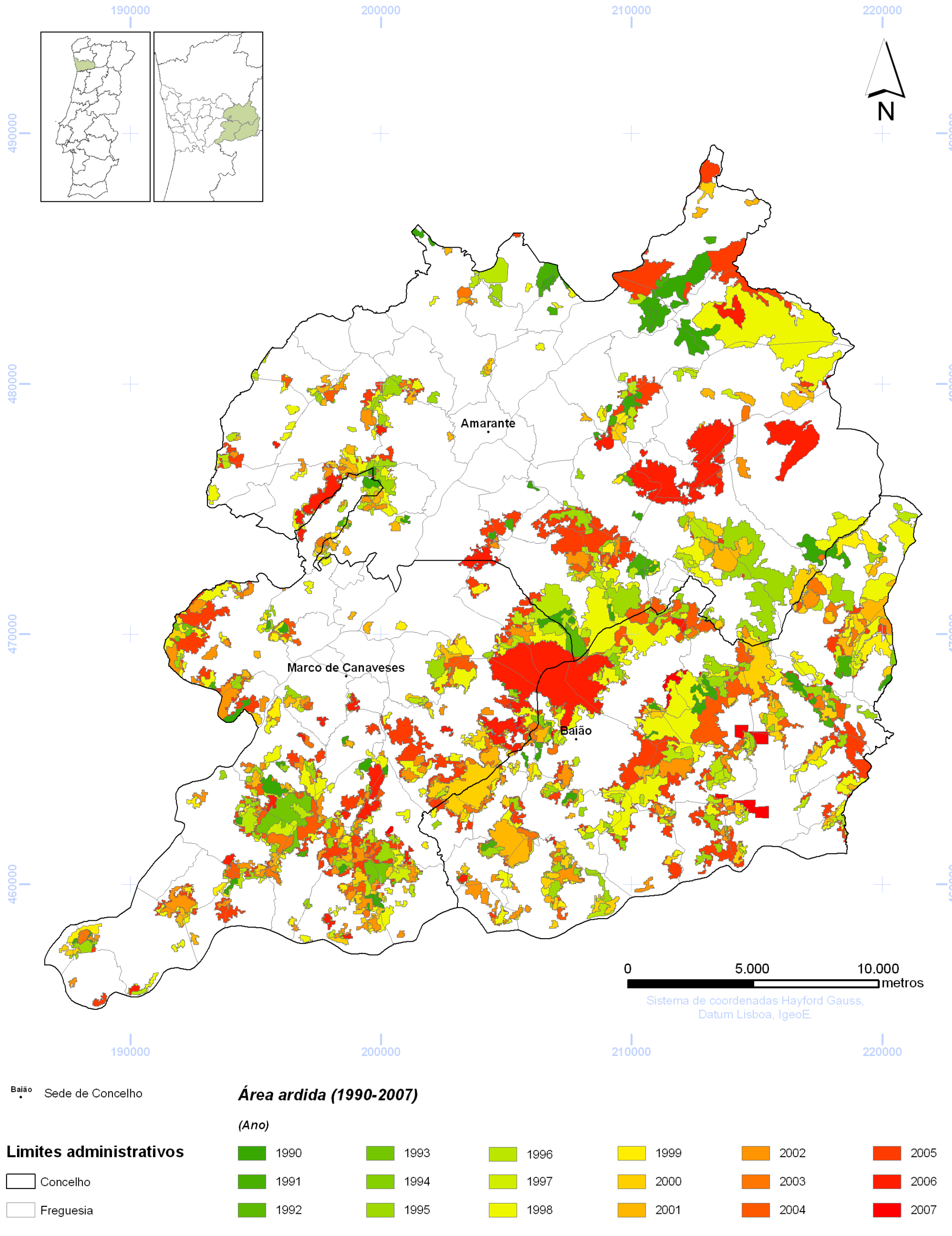


Figura 2. Carta das áreas ardidas no período 1990-2007 para o território do Baixo Tâmega.
(Retirado de Honrado *et al.*, 2009).

1.4. DESCRIÇÃO DETALHADA DA METODOLOGIA USADA NA DETERMINAÇÃO TAXONÓMICA DOS EXEMPLARES DE LÍQUENES RECOLHIDOS NO TERRENO

1.4.1. Recolha e processamento dos exemplares

A colheita dos espécimes foi efectuada com auxílio de canivete. Seleccionaram-se espécimes preferencialmente inteiros para posterior herborização. Os exemplares foram preservados em sacos de papel devidamente identificados e transportados para o laboratório para secagem ao ar, separação e identificação. O excesso de substrato foi cuidadosamente retirado dos espécimes colectados.

No laboratório procedeu-se à separação de todos os espécimes com base na observação das diferenças morfológicas mais evidentes. Uma vez isolados, os espécimes foram colocados em envelopes individuais que incluem informação sobre Data de Colheita, Local de Colheita (Freguesia, Concelho, Posição Geográfica), Colector, Fenologia e Reacções Químicas do Talo e Medula e restantes dados comuns a amostras de herbário.

1.4.2. Determinação taxonómica dos exemplares

1.4.2.1. Morfologia e Anatomia

Numa primeira fase de identificação dos géneros, recorreu-se a chaves dicotómicas de floras generalistas, designadamente a flora europeia de Clauzade & Roux (1985) e a flora do Reino Unido de Purvis *et al.* (1992). Sempre que disponíveis, consultaram-se monografias de géneros para a identificação até à espécie, nomeadamente Boqueras *et al.* (2003, 1999) para os géneros *Pertusaria* e *Ochrolechia*, Burgaz (2007) para o género *Cladonia*, Fos & Clerc (2000) para o género *Usnea* e Giralt *et al.* (2000) para o género *Buellia*. Os restantes nomes das espécies foram obtidos com base nas chaves dicotómicas da flora europeia mencionada e posteriormente actualizados de acordo com Hladun (2000-2007).

No processo de identificação recorreu-se a um estereomicroscópio para observação de inúmeras características morfológicas das quais se podem destacar a forma, cor e tamanho do talo; a presença de propágulos vegetativos e respectiva forma e distribuição no talo; a presença e tipo de rizinas; a presença de cifelas ou pseudocifelas, entre outras.

As características anatómicas do talo liquénico e respectivas estruturas reprodutoras foram observadas ao microscópio óptico com lentes objectivas até 100x, incluindo as características dos apotécios, forma, cor e tamanho dos esporos, bem como o número de esporos por asco. Todos os cortes foram executados "à mão livre", com lâmina de barbear. Todas as medições foram efectuadas em solução aquosa com recurso a uma lente ocular micrométrica.

1.4.2.2. Quimiotaxonomia

Para auxiliar o processo de identificação utilizaram-se os reagentes químicos habitualmente empregues em quimiotaxonomia liquénica, nomeadamente, solução de hidróxido de potássio a 10% (teste K), solução de hipoclorito de sódio ou lixívia comercial (teste C) e solução alcoólica de para-fenilenodiamina (teste P). Estes reagentes foram aplicados com o auxilio de um capilar na medula e/ou no córtex, consoante o indicado nas chaves utilizadas. Os testes medulares foram realizados mediante a remoção de uma pequena fracção do córtex com uma lâmina de barbear.

A alteração de cor resultante da aplicação destes reagentes é reveladora da presença de substâncias liquénicas com importância taxonómica, e foi devidamente anotada. Todas as reacções positivas foram indicadas com um sinal mais (+) seguido da indicação da cor resultante, as reacções negativas foram indicadas com um sinal menos (-). Teve-se particular atenção ao facto de algumas reacções só revelarem uma alteração de cor após um curto espaço de tempo pelo que se aguardou sempre alguns instantes até o registo do resultado da reacção.

Os testes químicos foram realizados em partes jovens do talo e efectuados sob o estereomicroscópio com luz branca para que alterações de cor do tipo amarelo não fossem consideradas falsos negativos.

Sempre que algum espécime apresentava dificuldades de identificação recorrendo apenas a caracteres morfológicos e anatómicos, foi realizada Cromatografica em Camada Fina de acordo com a metodologia proposta por Orange *et al*. (2001) para identificação de compostos químicos de importância taxonómica. Os extractos dos espécimes a identificar foram obtidos com acetona, e colocados em placas de alumínio 20x20 cm revestidas por sílica gel. Usaram-se dois padrões, atranorina e ácido norstíctico, presentes em *Platismatia glauca* e em *Pleurosticta acetabulum*, respectivamente, cujos extractos foram usados como controlo.

Foi traçada uma linha paralela à base da placa da qual distava 1 cm, nela foram colocados os extractos liquénicos com um espaçamento entre si de 1 cm recorrendo a um capilar de vidro. A colocação do extracto foi executada repetidas vezes até esgotar toda a solução. A cada amostra foi atribuído um número devidamente anotado no respectivo envelope de herbário. Uma outra linha foi traçada na placa a 2 cm do topo para assinalar o término da corrida do solvente.

Depois de colocados os extractos, as placas foram inseridas em duas tinas cromatográficas com os respectivos solventes: o solvente A (tolueno/1,4-dioxano/ácido acético, 180:45:5) e o solvente C (ácido tolueno/ácido acético, 170:30). Para facilitar a ascensão do solvente C uma das placas foi previamente colocada durante 10 minutos numa tina com atmosfera saturada de ácido acético. Após a migração dos solventes, as placas foram pulverizadas com ácido sulfúrico a 10% e colocadas numa estufa durante cerca de 10 minutos a 110 ^{o}C. Depois do aquecimento, as placas revelaram um leque de manchas cujas cores e percurso na placa são características de cada composto.

Entre os espécimes sujeitos a Cromatografia em Camada Fina estiveram todos os pertencentes aos géneros *Cladonia* e *Usnea*. A interpretação dos dados da cromatografia foi elaborada com base nas tabelas propostas por Orange *et al*. (2001).

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EPIPHYTE LICHEN DIVERSITY IN OAK WOODLANDS FROM ABOBOREIRA AND CASTELO MOUNTAIN (DOURO LITORAL, PORTUGAL)

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ABSTRACT

The lichen flora of Aboboreira and Castelo mountain is poorly known and based on incomplete and fragmentary observations with no lichenological studies ever published.

This study presents a catalogue of 70 species grouped in 32 genera. It was based on the survey of 65 trees, using traditional floristic methods. The epiphytic lichen flora was characterized based on different floristic and ecological affinities.

Aboboreira and Castelo mountain is dominated by epiphytic lichen species with larger distribution pattern (Boreal to Mediterranean distribution). Species with a broader distribution pattern are less ecologically dependent and can survive on many trees and microhabitats other than oak trunks.

Although presenting a high overall Diversity Index for Epiphytic Lichens (IDLE) value, some sites from Aboboreira and Castelo mountain show very low epiphytic lichen diversity, probably reflecting their young and recovering nature. Even through, the presence of a lichen community that has suffered general decline throughout Europe and the presence of some rare species such as *Parmelliela parvula*, stresses the need to develop management actions for this area. Therefore, Aboboreira and Castelo oakwoods are of high importance for epiphytic lichen conservation.

Keywords: Lichens, biodiversity, Portugal, *Quercus*, valoration.

INTRODUCTION

It is well known that the establishment of epiphytic lichens is restricted to well-structured natural woodlands, especially in the Mediterranean area (Fuertes *et al.*, 1996). The Portuguese territory has experienced a long history of replacement of these natural woodlands by large extensions of *Eucaliptus* and *Pinus* plantations, particularly in the northwest of the country, where Aboboreira and

Castelo mountain is situated. Fire is also an important agent of habitat disruption in the region and plays a major role in the definition of the landscape (Honrado & Vieira, 2009). Nevertheless, woodlands are still represented in the study area, with some being slowly recovering after recent agricultural abandonment and characterized by the presence of *Quercus robur* (*Rusco aculeati-Quercetum roboris*) followed by *Quercus pyrenaica* and *Quercus* x *andegavensis*. The consequences of this abandonment have not been fully estimated yet (Honrado & Vieira, 2009), but should have some effect over lichen vegetation, particularly epiphytes in recovering woodlands. As it happens with most part of the Portuguese territory, the lichen flora of Aboboreira and Castelo is poorly known and based on incomplete and fragmentary observations with no lichenological studies ever published. Jones (1999) reported the distribution and composition of *Nephroma laevigatum* communities in Portugal, including some of the most recent references for the northwest. It is widely recognized that assessments of forest biodiversity are essential if forest resources are to be effectively and sustainably managed (Lindenmayer *et al*., 2006). The vulnerability of epiphytic lichens to habitat disruption emphasises the importance of baseline studies in relatively undisturbed areas (Brodo *et al*., 2001) and integration of these group of species in forest monitoring programs. Therefore this study aims to provide useful baseline data on the epiphytic lichens from Aboboreira and Castelo mountain in order to extend the use of these organisms as ecological bioindicators in the study area.

MATERIAL AND METHODS

Aboboreira and Castelo (c. 105 km^2) is a mountain area reaching approximately 1000 m.a.s.l with a NE-SO orientation and surrounded by Ovelha, Ovil, Fornelo and Douro rivers (Cruz, 2004).

Since it is relatively close to the Atlantic Ocean, the climate is oceanic, with frequent fogs, abundant precipitation (AP: 1500-2000 mm) and an annual average temperature of 13^{o}C. However, summers are relatively dry, reflecting a (sub)Mediterranean influence, characteristic of the Iberian climate (Henrique *et al.*, 2004).

This study is based on a selection of 13 different oak wood sites (table 1). Within each site, five living oaks located at the centre of the woodland were randomly chosen, resulting in the analysis of a total of 65 trees. Each trunk was searched for species up to 2 meters high above ground. Species' occurrences higher up on the tree trunks or in crowns were not recorded since it was not our intention to produce a complete list of all epiphytic lichens but to provide a reference list for future monitoring programs, where species from the branches are usually neglected (Dietrich & Scheidegger, 1997). Host trees included specimens of *Quercus robur*, *Quercus pyrenaica* and *Quercus* x *andegavensis*. For each site, altitude, position and aspect were registered (table 1). Fieldwork was carried out in the middle of November.

Collected specimens were identified in the laboratory applying the usual stereomicroscopy and microscopy techniques, chemical spot tests and Thin-Layer Chromatography (TLC). Nomenclature follows Hladun (2000-2007). All specimens were kept in the Oporto University Herbarium (PO).

Table 1. Position (UTM), altitude (m), slope (degrees) and aspect of each studied sites.

	Site name	Position (UTM)	Altitude (m)	Slope (degrees)	Aspect
ABO1	Pardinhas	29TNF8463	500m	40	N
ABO2	Carvalho de Rei	29TNF8463	500m	30	N
ABO3	Carneiro	29TNF8663	550m	25	SW
ABO4	Zibreira	29TNF8965	900m	30	SW
ABO5	Zibreira	29TNF8965	900m	45	S
ABO6	Zibreira	29TNF8965	900m	30	SW
ABO7	Estucada	29TNF8663	750m	45	S
ABO8	Estucada	29TNF8663	750m	10	W
ABO9	Loivos do Monte	29TNF8662	700m	40	NE
ABO10	Bartioso	29TNF9162	500m	45	W
ABO11	Outeiro de Ante	29TNF8160	750m	30	S
ABO12	Outeiro de Ante	29TNF8160	750m	50	E
ABO13	Outeiro de Ante	29TNF8160	750m	45	S

Decomposition of the total amount of epiphytic lichen diversity (gamma diversity) into the components of mean diversity within samples (alpha diversity) and diversity among samples (beta diversity), was calculated according to the principle of additive partitioning of diversity into alpha and beta components (Veech & Crist, 2009).

Qualitative assessment of epiphytic lichens in the studied oak woods was based on the Diversity Index for Epiphytic Lichens (IDLE), developed by Aragón *et al.* (2008). This index incorporates species richness, species frequency and genera associated quality values, falling into five quantitative categories, namely very high (IDLE≥175), high (125≤IDLE<175), moderate (75≤IDLE<125), low (50≤IDLE<75) and very low (IDLE<50) diversity.

Species phytogeographic characterization was based on Wirth's system (Wirth, 1995) that brings data on both the latitudinal and the longitudinal variation of European lichen species distribution. The categories here presented were distributed in ten classes as suggested by the author. The ecological interpretation of the floristic-vegetation data is also based on the ecological indices proposed by Wirth (1995), namely hygrophily, photophily and nitrophily indices (table 2).

Table 2. Ecological affinities and phytogeography pattern recorded for each lichen species.

Trait	Description
Ecological affinities	
1. Hygrophily (H)	**Six classes**: 1: extremely hygrophytic (e-hyg); 2: very hygrophilous (v-hyg); 3: slightly hygrophytic (s-hyg); 4: meso-hygrophytic (mes); 5: slightly xerophytic (s-xer); 6: very xerophytic (v-xer).
2. Photophily (L)	**Five classes**: 1: very sciophytic (v-esc); 2: slightly sciophytic (s-esc); 3: moderately esciophytic/photophytic (m-esc); 4: slightly photophytic (s-pho); 5: very photophytic (v-pho).
3. Nitrophily (N)	**Five classes**: 1: anitrophytic (anit); 2: slightly nitrophytic (s-nit); 3: moderately nitrophytic (m-nit); 4: very nitrophytic (v-nit); 5: extremely nitrophytic (e-nit).
Phytogeography (P)	**Ten Classes**: Arct: Arctic; Arct-Med: from Arctic to Mediterranean; Bor-Med: from Boreal zone to Mediterranean; Bor-Smed: from Boreal to Submediterranean; Bor-Eur: from Boreal zone to Middle Europe; Eur-Smed: from Middle Europe to Submediterranean; Eur-Med: from Middle Europe to Mediterranean; Smed-Med: from Submediterranean to Mediterrarnean; Smed: Submediterranean; Med: Mediterranean.

RESULTS AND DISCUSSION

1. Diversity patterns

The present catalogue consists of 70 species (gamma diversity) grouped in 32 genera. The most abundant genera are *Pertusaria* (8 species), *Cladonia* (7 species), *Usnea* (6 species), *Parmelia* (4 species) and *Melanelia* (4 species). It is worth mentioning the fact that 24% of genera were represented by a single species. The dominant growth-form is foliose (49%), followed by crustose (33%) and fruticose (18%). In terms of associated photobiont, most species (83%) are green algae lichens, while only a few (17%) are cyanolichens. The preponderance (52%) of the lichen species were nitrogen-intolerant.

The number of species per sample ranged from 3 (ABO13) to 56 (ABO3), with a mean alpha richness of 23 species and a mean beta richness of 13 species (table 3) stressing the heterogeneity of the oakwoods in the studied area. Although species such as *Evernia prunastri* and *Parmelia sulcata* were present in all studied sites, the majority, namely *Heterodermia obscurata, Parmeliella parvula, Pannaria rubiginosa,* is exclusive of a few species richest sites, especially ABO1, ABO2 and ABO3.

Table 3. Additive partition of epiphytic lichen species diversity in Aboboreira and Castelo oakwoods.

	Additive Richness
Alpha	22.85
Beta	13.15
Gamma	70

Phytogeographically (figure 1), Aboboreira and Castelo mountain is dominated by epiphytic lichen species with a Boreal to Mediterranean distribution, followed in number by those with a Middle Europe to Mediterranean occurrence, and therefore with a large area of occurrence. It is evident from figure 1 that there are no Submediterranean to Mediterranean species present in the study area and that the less represented species are the ones with a more restricted distribution. Species with a broader distribution pattern are less ecologically dependent and can survive on many trees and microhabitats other than oak trunks.

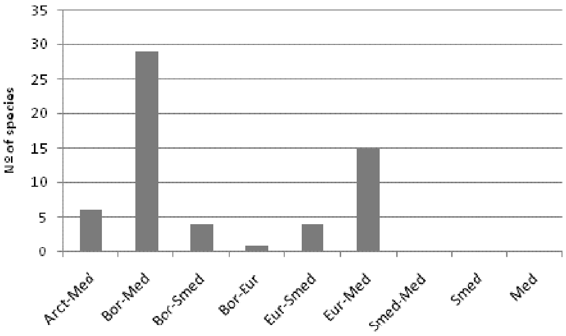

Figure 1. Phytogeographic pattern of epiphytic lichen in Aboboreira and Castelo.

Epiphytic lichens from Aboboreira and Castelo mountain tend to be slightly to very hygrophytic (figure 2a), confirming expectations from the knowledge of the climate of the area.

The majority of the species are slightly photophytic, with preference for light spaces but no direct radiation (figure 2b). These are followed by moderately sciophytic/photophytic species. The absence of high-light susceptible lichens is in conformity with our sites management history, since they present a reduced tree density and canopy cover, as a result of fire and logging.

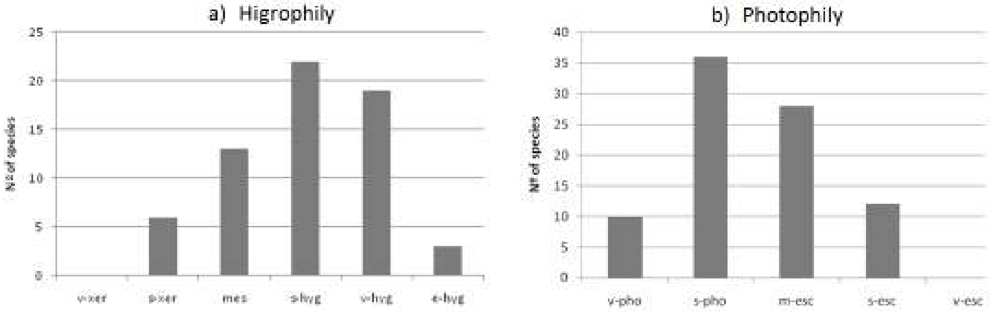

Figure 2. Ecological affinities of epiphytic lichen in Aboboreira and Castelo. a) higrophily, b) photophily.

2. Conservation value

The overall IDLE for the studied oakwoods was high (136), mainly due the presence of “old-growth” associated species *such as Collema subflaccidum, Degelia plumbea, Lobaria amplissima, L. pulmonaria, L. scrobiculata, Nephroma laevigatum, Pannaria mediterranea, P. rubiginosa, Parmelliela parvula*, *Peltigera collina, Sicta limbata* and *S. fuliginosa*.

Table 4. Frequency (F), Quality Value (IC) and Diversity Index for Epiphytic Lichens (IDLE) results for each of the study sites.

	ABO1	ABO2	ABO3	ABO4	ABO5	ABO6	ABO7	ABO8	ABO9	ABO10	ABO11	ABO12	ABO13	*Overall*
F	16	13.4	20.2	14	9.4	7.8	15.2	8.6	10.8	10	3	3.2	0.8	***10.75***
IC	85.2	74.4	121.8	63.4	30.4	32	85.6	43.4	56.2	51.2	11.4	22	3	***55.38***
IDLE	122	111	182	100	73	68	123	79	92	87	46	57	37	***136***

Although presenting a high overall IDLE value, some sites from Aboboreira and Castelo mountain show very low epiphytic lichen diversity (ABO11 and ABO13), table 4. Fire is one of the probable causes of lichen diversity impoverishment, since it leads to a reduction of tree cover and canopy density, and thus increased light incidence and decreased water availability, favoring species with lower ecological requirements. This would also explain the high representation of the heliophytic and nitrophytic *Physconia* and *Melanelia* species in the studied sites. Nevertheless, the presence of a lichen community that has suffered general decline throughout Europe as a consequence of air pollution and intensive forest management (Gauslaa, 1995), including some rare species such as *Parmelliela parvula* (reported here for the first time in northern Portugal and a second reference for the country), stresses the need to develop management actions for this area. Therefore it is our opinion that Aboboreira and Castelo oakwoods are of high importance for epiphytic lichen conservation.

CATALOGUE

Species are listed alphabetically with reference to the respective ecological indices (H = hygrophyly, L = photophyly) and phytogeographic pattern (P), whenever this information was available. Species marked with (**) are considered good indicators of “old-growth” forests (Coppins & Coppins, 2002; Rose & Coppins, 2002).

Buellia disciformis (Fr.) Mudd

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (1-2-3) ABO2 (1-2) ABO5-1

It seems to be a rather common species, widely distributed in Portugal and in northern Spain except for coastal localities (Giralt *et al*., 2000).

P: Boreal – Mediterranean; H: 3; L: 2-4

Caloplaca holocarpa (Hoffm.) Wade

On *Quercus robur, Quercus x andegavensis*, ABO1-3 ABO3-1

On bark and wood of many types of trees. Very frequent in Portugal.

P: Arctic – Mediterranean; H: 5; L: 3-4

Cetraria clorophylla (Willd.) Vainio

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1-2 ABO2 (1-2-3) ABO3 (1-2) ABO4-1 ABO5-1

Easily confused with *Platismatia glauca*, which differs in the presence of a darker underside towards the center.

P: Boreal – Submediterranean (mountains) – (Mediterranean.Mountains); H: 2-3; L: 2-5

Chrysothrix candelaris (L.) Laundon

On *Quercus robur, Quercus pyrenaica,* ABO1-1 ABO 4-1

On shaded bark of all kinds and occasionally on rock, widely distributed in rich, old forest, but also on roadside trees. Cosmopolitan species but absent in high mountains and deserts.

P: South Boreal – Mediterranean; H: 3; L: 2-4

Cladonia humilis (With.) J. R. Laundon

On *Quercus robur*, ABO3-1

P: Middle Europe – Mediterranean

Cladonia coniocraea (Florke ex Sommerf.) Sprengel

On *Quercus robur,* ABO1-1 ABO3 (1-2)

P: Boreal – Submediterranean – (Mediterranean); H: 3-4; L: 3-4

Cladonia cornuta (L.) Hoffm.

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (1-2-3)

P: Boreal

Cladonia fimbriata (L.) Fr.

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (1-3) ABO2 (1-3) ABO3-1 ABO4-1 AB5-1

Widespread and common species on humus and wood occurring in a wide variety of habitats.

P: (Arctic) – Boreal – Mediterranean; H: 3-5; L: 2-4

Cladonia pyxidata (L.) Hoffm.

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (1-3) ABO3-2

Cosmopolitan species with great ecologic amplitude.

P: Arctic – Mediterranean; H: 3-5; L: 3-4

Cladonia ramulosa (With.) Laudon

On *Quercus robur* ABO1-2

P: Middle Europe - Submediterranean

Collema fasciculare (L.) Weber ex Wigg.

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (2-3)

Always with numerous apothecia obscuring thallus surface.

P: Middle Europe - Mediterranean

Collema subflaccidum Degel.

** On *Quercus pyrenaica, Quercus x andegavensis*, ABO1-3

Degelia plumbea (Lightf.) P. M. Jorg & P. James

** On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (2-3) ABO3-1 ABO4 (2-3) ABO5-2

In the study area the individuals usually present little marginal squamules similar to isidia of *D. atlantica*.

P: Boreal/Atlantic – Middle Europe/Atlantic – Mediterranean; H: 2; L: 2-4

"Dendriscocaulon umhausense" (Auersw.) Degel.

On *Quercus robur, Quercus pyrenaica,* ABO1 (2-3) ABO2-2 ABO4-1

Cephalodia of *Lobaria amplissima*.

Evernia prunastri (Huds.) M. Choisy & Werner

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (1-2-3) ABO2 (1-2-3) ABO3 (1-2) ABO4 (1-2) ABO5 (1-2-3)

Has been used as an additive in bread and is commercially important in the perfume industry. Have is optimal in Quercus-Fagetea communities. Appear in all the study area.

P: Boreal – Mediterranean; H: 2-4; L: 3-5

Flavoparmelia caperata (L.) Hale

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (1-2-3) ABO2 (1-2-3) ABO3 (1-2) ABO4 (1-2) ABO5-1

P: Middle Europe – Mediterranean; L: 4

Flavoparmelia soredians Nyl.

On *Quercus pyrenaica,* ABO2-1

Frequent in Portugal.

Heterodermia obscurata (Nyl.) Trevis.

On *Quercus pyrenaica, Quercus x andegavensis*, ABO1-3

On barks or hardwoods, occasionally on shade rocks.

Hypogymnia physodes (L.) Nyl.

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1-1 ABO2 (1-2-3) ABO3 (1-2) ABO4-1 ABO5-1

Specie that colonize a vast variety of substratum. Toxitolerant to SO_2.

P: Arctic – Mediterranean; H: 3-4; L: 3-4

Hypogymnia tubulosa (Schaerer) Havaas

On *Quercus robur, Quercus pyrenaica,* ABO1-1 ABO2-1 ABO3-2

Appears with more frequency in high mountains and is favoured by open and extremely illuminated forests. Can be confused with *Hypogymnia physodes*, which differs in having lip-shaped as opposed to globular soralia.

P: Boreal – Mediterranean; H: 3; L: 3-5

Lecanora chlarotera Nyl.

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (1-2-3) ABO2-1 ABO5-1

P: Boreal - Mediterranean

Lecanora pallida (Schreb.) Rabenh.

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1-2 ABO2-2 ABO5-1

P: South Boreal – Mediterranean/Mountains; H: 2-3; L: 2-3

Lepraria incana (L.) Ach.

On *Quercus robur,* ABO1 (1-2)

P: Boreal – Middle Europe – Submediterranean; L: 4

Leptogium brebissonii Mont.

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1-3 ABO3-1 ABO4-1

Lobaria amplissima (Scop.) Forss.

** On *Quercus robur, Quercus x andegavensis*, ABO3-2 ABO5-2

With frequent cephalodia – "*Dendriscocaulon umhausense*".

P: Boreal/Atlantic – Middle Europe/Sulatlantic – Mediterranean/Mountains; H: 2; L: 3-4

Lobaria pulmonaria (L.) Hoffm.

** On *Quercus pyrenaica, Quercus x andegavensis*, ABO1-3

It almost disappeared in North and Middle Europe. One cause for this is it extreme sensibility to air pollution but Walser (2004) showed that another possible cause is the limited dispersal of vegetative propagules and the absent of morphological adaptations for dispersal, therefore regeneration should only be possible in short distances.

P: Boreal – Mediterranean/Mountains; H: 1-2; L: 4

Lobaria scrobiculata (Scop.) DC. in Lam & DC.

** On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (1-2-3) ABO2-1 ABO3 (1-2) ABO4 (1-2) ABO5 (1-2)

Good indicator of moderated conditions of temperature and humidity.

P: Boreal – Mediterranean/Mountains; H: 1-2; L: 3

Melanelia elegantula (Zahlbr.) Essl.

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO2-3 ABO3-1

Moderately tolerant to air contamination, it often behaves as an opportunistic species.

P: Middle Europe – Mediterranean; H: 4; L: 4

Melanelia exasperatula (De Not.) Essl.

On *Quercus pyrenaica, Quercus x andegavensis*, ABO2-2

Highly tolerant to air pollution. Characteristic of the *Xanthorion parietinae* alliance and a pioneer species in tree colonization.

P: Boreal – Mediterranean; H: 4-5; L: 4-5

Melanelia glabratula (Lamy) Essl.

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (1-3) ABO2 (1-2) ABO3 (1-2) ABO4 (1-2) ABO5 (1-3)

P: Boreal – Mediterranean

Melanelia subargentifera (Nyl.) Essl.

On *Quercus robur* ABO5-1

P: Boreal – Midlle Europe – Mediterranean; L: 4-5

Nephroma laevigatum Ach.

** On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (1-2-3) ABO2 (1-2) ABO3 (1-2) ABO4 (1-2) ABO5 (1-2)

A very common species in Portugal.

P: Boreal – Midlle Europe – Mediterranean; H:2; L: 2-3

Normandina pulchella (Borrer) Nyl.

On *Quercus pyrenaica, Quercus x andegavensis*, ABO1-3 ABO3-1

P: Middle Europe – Mediterranean/Subatlantic; H: 2-3; L: 4

Ochrolechia szatalaeuris Verseghy

On *Quercus x andegavensis*, ABO4-2

Has preference for *Quercus pyrenaica* but can be found in other phorophytes such as *Pinus sylvestris* (Boqueras *et al.*, 1999).

P: Boreal – Midlle Europe – Mediterranean; L: 3

Pannaria mediterranea Tavares

** On *Quercus robur,* ABO1-1

Collected by Tavares, in 1959, between Castelo de Vide and Marvão and later described as a new species.

Pannaria rubiginosa (Ach.) Bory

** On *Quercus pyrenaica* ABO1-3

Occurs on mossy bark and stone in oceanic regions.

P: South Boreal/Atlantic – Middle Europe (Atlantic) – Mediterranean/Mountains (Subatlantic); H: 2; L: 2-4

Parmelia quercina (Willd.) Vainio

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1-1 ABO2 (1-2-3) ABO4-2

P: Midlle Europe – Mediterranean; L: 4-5

Parmelia saxatilis (L.) Ach.

On *Quercus pyrenaica,* ABO4-1

Widespread and especially common at higher altitudes and latitudes. Frequent on bark and acid stone.

P: Arctic – Middle Europe – Mediterranean (Mountains); H: 3; L: 3-4

Parmelia sulcata Taylor

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (1-2-3) ABO2 (1-2-3) ABO3 (1-2) ABO4 (1-2) ABO5 (1-2-3)

P: Arctic – Mediterranean; H: 3-5; L: 4

Parmelia tiliacea (Hoffm.) Ach.

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (2-3) ABO2-1 ABO3-1 ABO4 (1-2) ABO5-1

Widespread on roadside, avenue trees and rocks.

P: Middle Europe – Mediterranean; H:4; L: 4-5

Parmeliella parvula P.M. Jorg.

** On *Quercus pyrenaica* ABO1-3

Grows on mossy bark in Mediterranean Atlantic regions. Rare specie and very sensible to habitat alterations. Observed only once in our study.

Parmotrema chinense (Osbeck) Hale & Ahti

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (1-2-3) ABO2 (1-2-3) ABO3 (1-2) ABO4 (1-2) ABO5 (1-2)

Is the *Parmotrema* species more frequent in Iberian Peninsula.

P: Middle Europe/Subatlantic – Mediterranean (Mountains); H: 4; L: 4

Peltigera collina (Ach.) Schrader

** On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1-3 ABO3-1 ABO5-2

Characteristic of *Lobarion* communities. Distinguished from other species of the same genera by the presence of soredia.

P: Boreal – Middle Europe (Subatlantic) – Mediterranean/Mountains

Pertusaria albescens (Huds.) M. Choisy & Werner

On *Quercus pyrenaica, Quercus x andegavensis*, ABO1 (1-3) ABO2-2

A very common lichen in Portugal. Tolerant to high levels of air pollution.

P: (South) Boreal – Mediterranean; H: 3-4; L: 3-4

Pertusaria amara (Ach.) Nyl.

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (1-2-3) ABO2 (1-2) ABO3 (1-2) ABO4 (1-2) ABO5 (1-2)

With white soralia of bitter taste. Very frequent in our study site.

P: (South) Boreal – Mediterranean; H: 4; L: 3-4

Pertusaria caesioalba (Flot.) Nyl.

On *Quercus pyrenaica,* ABO2-1

Morphologically related to *P. paramerae* but with different chemistry (Boqueras & Llimona, 2003).

Pertusaria coccodes (Ach.) Nyl.

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (1-2-3) ABO3-1 ABO4-2

Appears at a wide diversity of trees, preferably in open areas of forests (Boqueras & Llimona, 2003).

P: (South) Boreal – Middle Europe (Subatlantic) – Mediterranean; H: 3; L: 3-4

Pertusaria flavida (DC.) Laundon

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (2-3) ABO2 (1-3)

Common in the mountain areas of the Iberian Peninsula. This species is easily identified by the KC+ and C+ orange reaction of the thallus and the presence of sorediiferons isidia (Boqueras & Llimona, 2003).

P: (South Boreal) – Middle Europe/Subatlantic – Mediterranean/Mountains; H: 2-3; L: 3-4

Pertusaria hemisphaerica (Floerk) Erichsen

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (1-2-3) ABO4-2

P: South Boreal – Middle Europe/Subatlantic – Mediterranean; H: 2-3; L: 3-4

Pertusaria hymenea (Ach.) Schaerer

On *Quercus pyrenaica, Quercus x andegavensis*, ABO1-3 ABO4 (1-2)

P: Middle Europe (Atlantic) – Mediterranean/Subatlantic; H: 2; L: 3

Pertusaria pertusa (Weigel) Tuck.

On *Quercus pyrenaica, Quercus x andegavensis*, ABO1 (1-3) ABO4-2

It is frequent in oak forests and in the mountain region forests, in sunny conditions (Boqueras & Llimona, 2003).

P: (South Boreal) – Middle Europe (Subatlantic) – Mediterranean (Mountains); H: 2-3; L: 3-4

Phylictis argena (Sprengel) Flotow

On *Quercus robur, Quercus x andegavensis*, ABO1 (1-3) ABO5 (1-2)

P: South Boreal – Mediterranean; H: 3-4; L: 3-4

Physconia distorta (With.) Laundon

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (2-3) ABO2 (1-2) ABO3-1 ABO4 (1-2) ABO5 (1-2)

P: South Boreal – Mediterranean; H: 5; L: 4-5

Physconia enteroxantha (Nyl.) Poelt

On *Quercus pyrenaica, Quercus x andegavensis*, ABO1-3

P: Boreal – Mediterranean; H: 4; L: 4

Physconia perisidiosa (Erichsen) Moberg

On *Quercus pyrenaica, Quercus x andegavensis*, ABO1-3

P: Boreal – Middle Europe – Mediterranean/Mountains

Platismatia glauca (L.) Culb. & C. Culb.

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO2-1 ABO3 (1-2) ABO4 (1-2)

P: Boreal – Middle Europe – Mediterranean/Mountains

Punctelia borreri (Sm.) Turn.

On *Quercus robur,* ABO3-1

Punctelia subrudecta (Nyl.) Krog

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (1-3) ABO2-1 ABO3-1 ABO4-1

P: Middle Europe – Mediterranean (Subatlantic); H: 4-5; L: 4

Ramalina calicaris (L.) Fr.

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1-2 ABO2-1 ABO4-2 ABO5-1

Ramalina farinacea (L.) Ach.

On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (1-2-3) ABO2 (1-2-3) ABO3 (1-2) ABO4 (1-2) ABO5-1

On all kind of trees and shrubs, rarely on rocks, in regions with a mild, humid climate (Brodo *et al*., 2001).

P: Boreal – Mediterranean; H: 2-5; L: 2-5

Ramalina fastigiata (Pers.) Ach.

On *Quercus robur,* ABO1 (1-2)

P: South Boreal – Mediterranean; L: 4-5

Sticta fuliginosa (Hoffm.) Ach.

** On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (2-3) ABO3-1 ABO4-2

P: Middle Europe - Mediterranean

Sticta limbata (Sm.) Ach.

** On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (1-2-3) ABO2-1 ABO3 (1-2) ABO5-1

Epiphytic specie sensible to low levels of air pollution. Declining in Europe but still frequent in Portugal and in our study area.

P: Middle Europe – Mediterranean/Atlantic; H: 2; L: 2-4

Usnea cornuta Korber

On *Quercus robur, Quercus pyrenaica,* ABO1 (1-2) ABO2-1 ABO3-1

Grows on wood, bark or rocks, usually in well-lit open situations. It is more frequent on branches, even though it can occur on in the trunk holes of numerous phorophytes (Fos & Clerc, 2000).

P: Middle Europe – Submediterranean

Usnea florida (L.) Weber ex Wigg.

** On *Quercus robur, Quercus pyrenaica, Quercus x andegavensis*, ABO1 (1-2-3) ABO2 (1-2) ABO3-2

In forests with frequent fog and without air contamination. *Usnea subfloridana* is closely related, but has abundant soredial isidia, and apothecia are scarce.

P: Middle Europe – Submediterranean; H: 2

Usnea glabrescens (Nyl. ex Vainio) Vainio

On *Quercus robur,* ABO3-2

P: Boreal – Middle Europe

Usnea rubicunda Stirton

On *Quercus robur,* ABO1-2

Photophilous and hygrophilous, common in areas with an oceanic climate and in ancient woodlands.

P: Middle Europe (Atlantic) – Mediterranean; H: 3; L: 2-4

Usnea wasmuthii Rasanen

On *Quercus pyrenaica* ABO2-1

Has its optimum in the southwestern part of the Iberian Peninsula (Fos & Clerc, 2000).

Usnea wirthii Clerc

On *Quercus pyrenaica, Quercus x andegavensis*, ABO1-3 ABO4-1

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RESTORATION OF EPIPHYTIC LICHEN DIVERSITY IN NORTHERN PORTUGAL BURNED OAK WOODS

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ABSTRACT

Fire ecology and post-fire secondary succession of higher plants has been extensively studied, yet little work has been done to understand the impact of fire on the diversity of epiphytic lichens. In this study we examined burned oak woodlands in 13 sites with 3, 8, 13, 18 after fire and unburned forests, to document colonization by lichens. Species response to disturbance depends in part on their life history, therefore, we studied the association between post-fire recovering and a few life history characteristics (ecological, vegetative and reproductive traits) for lichens in Aboboreira and Castelo mountain, North Portugal.

After a strong disturbance such as fire, the destruction of very specific habitats, present only in mature ecosystems, produces the loss of species with a restricted ecology, occurring only in those habitats. New habitat conditions resulting from fire were more favorable for lichens with broader ecological amplitude, more tolerance to intense light and water loss. Only the best preserved forests presented well established sciophytic communities of epiphytic lichens.

Composition of dominant species remained relatively constant followed the fire event and almost every lichen species appeared 3 years after fire remained indefinitely, since they were also present in old forests. Analyzing these results we presume that the source of propagules or spores were the neighboring species around the unburned substrates.

Keywords: Epiphytic lichens, post-fire recolonization, *Quercus* woodland, life-history traits, biodiversity, disturbance.

INTRODUCTION

Fire plays a central role in the shaping of the landscape along the Portuguese territory. It is known to determine forest community structure, species composition, and affect diversity and natural selection (Trabaud, 1994). Fire is an integral part of the forest management process, including oakwoods, since it clears away woody undergrowth and favors the growth of oaks by avoiding canopy closure which allows light to reach the ground (Edgin *et al.*, 2003). Most current studies of fire ecology and post-fire secondary succession are related to higher plants, showing, for instance, how species life histories determine their response and can predict post-fire community patterns (Gracia *et al.*, 2002; Reemts & Hansen, 2008). Considerable work has clarified the importance of various aspects of forest integrity for epiphytic lichens, but in general, the impact of fire on the diversity of epiphytic lichens has been ignored, despite the fact that these organisms are an important part of the ecosystem and its nutrient cycles providing substrate for later successional species, microhabitats, nutrient cycles and food for herbivores (Nash III, 1996). More importantly for recovering ecosystems, many lichen species present cyanobacterial photobionts and are therefore important in nitrogen cycling (Nash III, 1996). Past studies have documented the effects of fire and eventual recovery on soil cryptogams and crustose species in the desert (Garty, 1990, 1992; Johansen *et al.*, 2005). Other studies have investigated the effects of fire and post-fire restoration of lichen communities in boreal forests (Johansen, 2008), tropical forests (Mistry, 1998; Kuusinen, 1996; Wolseley & Hudson, 1997) or in the American continent (Romagni & Gries, 1997). Johansson *et al.* (2006) studied the habitat preference, growth form, vegetative dispersal and population size of lichen species along a wildfire severity gradient in Minnesota (USA) and concluded that pos-fire colonization rates are higher for lichens with high regional population size and dead wood preference. Research conducted in terricolous habitats has demonstrated that lichens cannot survive fire and reveal poor recovery ability (Kumpula *et al.*, 2000; Schulten, 1985).

In the Mediterranean region, the limitation in the literature is even more obvious, with few exceptions. Longán *et al.* in 1999 studied the colonization of epiphytic lichens from different phorophytes in Parque Natural de Sant Llorenç de Munt y Sierra de l'Obac (NNW de Barcelona) and observed that completely burned areas had a low lichen diversity concluding that the species with the greatest colonization success were those that colonize a wide variety of substrates. In 2001, Fos *et al.* studied post-fire colonization in the burned barks of cork-oaks (*Quercus suber* L.) and observed that specific richness and coverage of the epiphytic communities reflected differences between areas. These differences were related with both the proximity to undisturbed areas and the bioclimatic characteristics of the forests.

Although fires are recurrent in northern Portugal, there are no studies of which we are aware that document the effects of fire on local epiphytic lichen diversity. Lichens cannot survive fire, and many studies document their poor recovery capacity after burning due to their slow growth rate (Ketner-Oostra *et al.*, 2006; Kumpula *et al.*, 2000; Mistry, 1998; Schulten, 1985). Knowledge of the effect of fire on epiphyte lichens and their re-establishment could provide basic information for the future development of bioindication methods in Portuguese oak woods. According to Wolf *et al*. (2000) a good strategy for understanding and predicting the effects of fire on lichen vegetation is to study the effects on functional traits, due to their simplicity, and both time and cost reduction. Therefore, the aims of this paper are (1) to provide new data on the response of epiphytic lichen communities to fire in Portuguese oak woods in terms of both species richness and composition (2) to analyze the patterns or trends in the relative importance of various lichen traits along a post-fire gradient.

MATERIALS AND METHODS

Study area and sampling design

The study sites are located in the southeast region of the Marão Natural Park in Aboboreira and Castelo, Baião, Portugal. Aboboreira and Castelo mountain extend northeast to southwest in an area of 105 km^2 and reach a maximum elevation of approximately 1000 m a.s.l (Cruz, 2004).

The climate in this area is oceanic with frequent fogs, abundant precipitation (AP: 1500-2000 mm) and an annual average temperature of 13^{o}C. However, summers are relatively dry, reflecting a (sub)Mediterranean influence, characteristic of the Iberian climate (Henrique *et al.*, 2004).

In order to assess epiphytic lichen post-fire recovery in the study area and its main ecological influencing factors, we selected 13 sites. For the selection of the sites we used a dataset of burnt areas (burnt patches with more than 5 ha) between 1990 and 2007, in combination with a land cover map, for the location of oak patches.

The 13 selected sites were classified into five groups representing sequential years passed since last fire occurrence: 1) burned 3 years ago; 2) burned 8 years ago; 3) burned 13 years ago; 4) burned 18 years ago and 5) presumably unburned forests (no registry of fire since 1990).

In each site, five healthy trees were randomly selected among the population, summing up a total of 65 selected trees. Each trunk was searched for species up to 2 meters high above ground. Species' occurrences higher up on the tree trunks or in crowns were not recorded since it was not our intention to produce a complete list of all epiphytic lichens but to provide a reference list for future monitoring programs, where species from the branches are usually neglected (Dietrich & Scheidegger, 1997).

The first data set consisted of lichen species richness which was originally sampled for an inventory of epiphytic lichens from Aboboreira and Castelo mountain (Cruz *et al.*, 2009). A second lichen data set was obtained by calculating lichen species frequency in each site (number of trees out of five presenting the species). We decided to evaluate species frequency because it can define a species' performance at different environmental situations: the most frequent species in a specific area is more likely to be close to its optimum for a given environmental factor, than a species with a lower frequency. Host trees included specimens of *Quercus robur*, *Quercus pyrenaica* and *Quercus* x *andegavensis*. When species could not be identified in the field, specimens were collected for further identification in the laboratory (microscopy and thin-layer chromatography). Nomenclature follows Hladun (2000-2007). All specimens were kept in the Oporto University Herbarium (PO).

A third data set consisted of a few field variables from each site, such as canopy density (%), tree Diameter Breast Height or DBH (cm) and tree cover (%) known to reflect changes in the forest structure, besides altitude (m), slope (degrees) and aspect .

The geographic location of the studied sites, and respective field variables are presented in table 1.

Table 1. Position (UTM), altitude (m), aspect, slope (degrees), DBH (cm), tree cover (%) and canopy density (%) of the 12 studied sites.

Post-fire class	Site name	Position (UTM)	Altitude (m)	Slope (degrees)	Aspect	Tree cover (%)	Canopy density (%)	DBH (cm)
Unburned	Pardinhas	29TNF8463	500	40	N	70	70	30
	Carvalho de Rei	29TNF8463	500	30	N	80	80	30
	Carneiro	29TNF8663	550	25	SW	70	70	30
18 years	Zibreira	29TNF8965	900	30	SW	60	20	15
	Zibreira	29TNF8965	900	45	S	40	20	10
	Zibreira	29TNF8965	900	30	SW	50	50	15
13 years	Estucada	29TNF8663	750	45	S	85	85	30
	Estucada	29TNF8663	750	10	W	70	70	20
8 years	Loivos do Monte	29TNF8662	700	40	NE	70	70	20
	Bartioso	29TNF9162	500	45	W	60	60	20
3 years	Outeiro de Ante	29TNF8160	750	30	S	70	70	20
	Outeiro de Ante	29TNF8160	750	50	E	60	60	15

Data analysis

We compiled data on reproductive, vegetative and ecological traits for each of the 70 lichen species found in the study area (table 1). The most important data source for reproductive and vegetative traits was Stofer (2006). Ecological traits were based on the hygrophytic and photophytic indices proposed by Wirth (1995). Determinations for the trait "fertility" were based on expert opinion. Reproductive, vegetative and ecological traits are cataloged in table II, annex I.

For each class of each trait (table 2) relative species richness (called 'relative richness' in the following for convenience) was calculated as follows: first the number of species in all classes of a certain trait was summed for each of the 13 sites. Then the species numbers of each class of that trait was divided by the referred sum. This provided relative richness for each trait class in each site.

Qualitative assessment of epiphytic lichens in the studied oak woods was based on the Index of Epiphytic Lichen Diversity (IDLE), developed by Aragón *et al.* (2008).

Exploratory analysis of the lichen data set showed that one of the sampled sites (3 years since fire) was very different from all other sites and was therefore excluded from all the analyses.

Table 2. Vegetative, reproductive and ecological traits and trait class assigned to each lichen species.

Trait	Description
Vegetative traits	
1. Growth form	**Five classes:** cru: crustose; flad: foliose-adpressed flas: foliose-ascendant frer: fruticose-erect frpe: fruticose-pendulous
2. Photobiont	**Two classes:** cya: cyanobacteria; gre: green algae
Reproductive traits	
3. Fertility	**Two classes:** mfer: mostely fertile; mste: mostely sterile
4. Propagules	**Two classes:** sor: soredia; isi: isidia
Ecological traits	
5. Hygrophytic	**Six classes:** e-hyg: extremely hygrophytic; v-hyg: very hygrophytic; s-hyg: slightly hygrophytic; m-hyg: meso- hygrophytic; s-xer: slightly xerophytic; v-xer: very xerophytic
6. Photophytic	**Five classes:** v-sci: very sciophytic; s-sci: slightly sciophytic; m-pho: moderately sciophytic/photophytic; s-pho: slightly photophytic; v-pho: very photophytic.

The hypothesis of changes in absolute and relative richness of trait classes with time since fire was tested by Analysis of Variance (ANOVA) in SPSS Statistics 7.0. In a first series of ANOVAs, the post-fire gradient was considered as a fixed factor with five levels. If the overall effect of the fire gradient was at least marginally significant (probability of Type I error set at 0.1%), Tukey HSD procedure was applied for pair wise comparisons of means of species richness between levels of the post-fire gradient. In a second series of ANOVAs the post-fire gradient was considered as an ordered factor in order to test for linear trends of the post-fire gradient on relative richness. By this procedure, it was possible to detect significant trends in the data which were not detected by pair wise comparisons. ANOVAs assumption of homogeneity of variances was tested with Levene's test and of normality of the residual distribution checked graphically with normal probability plots. In such cases of heteroscedasticy and/or non-normality of the residual distributions, Kruskall-Wallis was used as a nonparametric alternative to ANOVA (see table 4).

In order to detect the extent of differences in both species richness and composition explained by fire, a Redundancy Analysis (RDA) was performed in CANOCO 4.5 (ter Braak & Smilauer, 2002), using time-since-fire as the only explanatory variable, as described by (Leps & Smilauer, 2003). This is a constrained ordination technique based on principal components analysis that, in a joint analysis of two data sets, assesses the degree to which they covariate. Exploratory analysis of the lichen data set showed a relatively short length of the compositional gradient suggesting the use of linear rather than unimodal models (Leps & Smilauer, 2003). Statistical significance of the constrained ordination axis was tested by Monte-Carlo randomization F-test with 499 unrestricted permutations.

RESULTS

For all species, a significant overall effect of fire was found on lichen species richness and a significant linear trend with increasing species richness along the post-fire gradient (figure 1a). Results show an increasing Index of Epiphytic Lichen Diversity (IDLE) along the post-fire gradient as well. In the first years following fire, species richness is the lowest (table 3) with 17 species of epiphytic lichens (12 foliose, 3 fruticose and 2 crustose) being able to rapidly colonize the tree trunks. Recently burned oak woods are therefore characterized by a very low diversity (IDLE = 31) of epiphytic lichens. Species progressively recolonize tree trunks and eight years passed since the last fire, the number of species stabilizes with little fluctuations at intermediate stages of recovery (13 and 18 years since last fire). Epiphytic lichen diversity at these stages should be considered moderate (IDLE = 83). Species richness reaches the highest value of 56 species (26 foliose, 16 fruticose and 14 crustose) in unburned oak woods, including twelve exclusive species, such as *Heterodermia obscurata, Parmeliella parvula* and *Pannaria rubiginosa*. Unburned oak woods are thus characterized by high values of IDLE (table 3). Only a small part (20%) of total species found in Aboboreira and Castelo oak woods was present in all post-fire recovering stages, including *Evernia prunastri*, *Flavoparmelia caperata*, *Hypogymnia physodes*, *Lobaria scrobiculata*, *Nephroma laevigatum*, *Parmelia sulcata*, *Parmotrema chinense* and *Pertusaria amara* (figure 1b-i). The species with the greatest colonization success was *Parmelia sulcata*, since it occurred in more than 50% of the trees of every studied oak woods. Although very frequent in unburned oak woods, a significant decrease in the frequency of *Lobaria scrobiculata* occurred 18 years after fire (figure 1e) contrasting with a significant increase of *Hypogymnia physodes* (figure 1d).

Table 3. Mean Species Richness (MSR), Total Species Richness (TSR) and Index of Epiphytic Lichen Diversity (IDLE) for the five post-fire classes.

	Post-fire classes				
	3 years	**8 years**	**13 years**	**18 years**	**Unburned**
MSR	13	22	25	20	36
TSR	27	29	34	33	56
IDLE	31	91	87	87	166

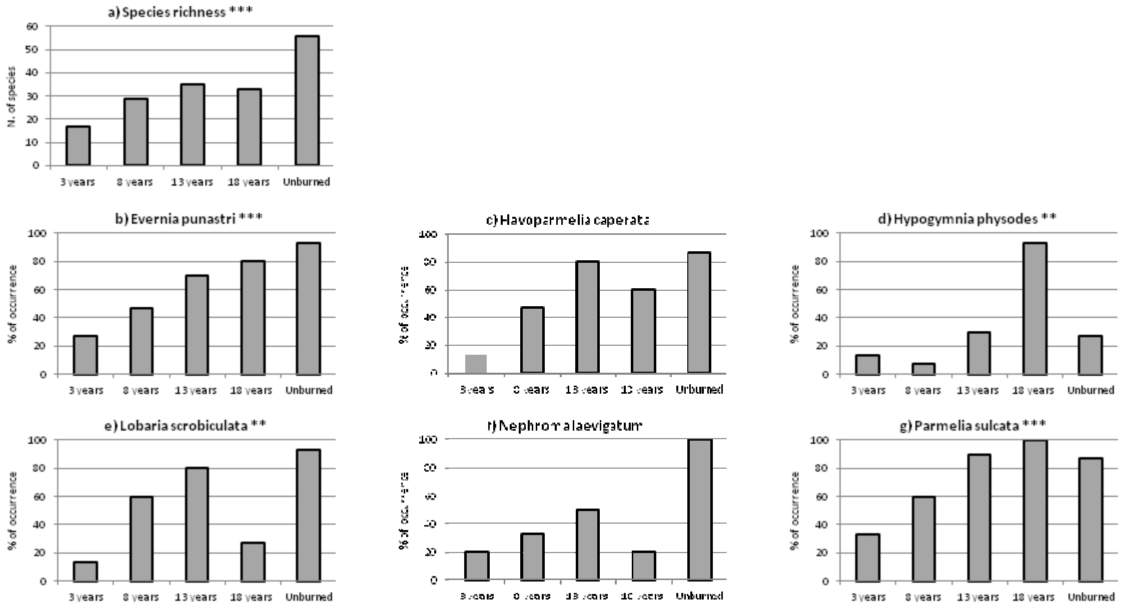

Figure 1. (a) Species richness and frequency (%) of (b) *Evernia prunastri;* (c) *Flavoparmelia caperata;* (d) *Hypogymnia physodes; (e) Lobaria scrobiculata; (f) Nephroma laevigatum;* (g) *Parmelia sulcata;* (h) *Parmotrema chinense;* and (i) *Pertusaria amara* on *oak* trunks at each site. Significative differences detected by ANOVA are signaled with *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

The relationships between the post-fire gradient and relative richness of trait classes as revealed by analyses of variance are summarized in table 4.

Table 4. Effects of the fire gradient on relative richness of trait classes as revealed by ANOVA (a) and Kruskal-Wallis (b) tests. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$. **§** is for second ANOVA series.

Traits	Trait class	F	§Trend	§Sign of trend
Growth form	Crustose	0.063* (b)		
	Foliose-adpressed	0.056* (b)		
	Foliose-ascendant	0.166 (b)		
	Fruticose-erect	0.647(a)		
	Fruticose-pendulous	0.219 (b)		
Fertility	Mostly Sterile	1.651(a)		
	Mostly Fertile	0.66(a)		
Photobiont	Green algae	3.723*(a)	*	-
	Cyanobacteria	3.723*(a)	*	+
Propagules	Soredia	0.394 (b)		
	Isidia	0.394 (b)		
Photophylly	Slightly sciophytic	4.058*(a)	**	+
	Moderately sciophytic/photophytic	0.188 (b)		
	Slightly photophytic	6.964**(a)	**	-
	Very photophytic	0.066* (b)		
Hygrophylly	Very hygrophytic	9.226**(a)	**	+
	Slightly hygrophytic	0.499 (b)		
	Meso- hygrophytic	0.059* (b)		
	Slightly xerophytic	0.91 (b)		

Table 5. Results from PCA analysis. (a) Eigenvalues and cumulative percentage of variance explained by axes; (b) Inter set correlations of environmental variables with axes.

		Axis 1	**Axis 2**
a)	Eigenvalue	0.327	0.169
	Cumulative % variance explained	32.7	49.6
b)	Fire	0.59	0.52
	Altitude	-0.63	0.41
	Slope	-0.12	-0.20
	Canopy density (canopy)	0.59	-0.11
	DBH	0.80	-0.07
	Tree cover (cover)	0.39	-0.36

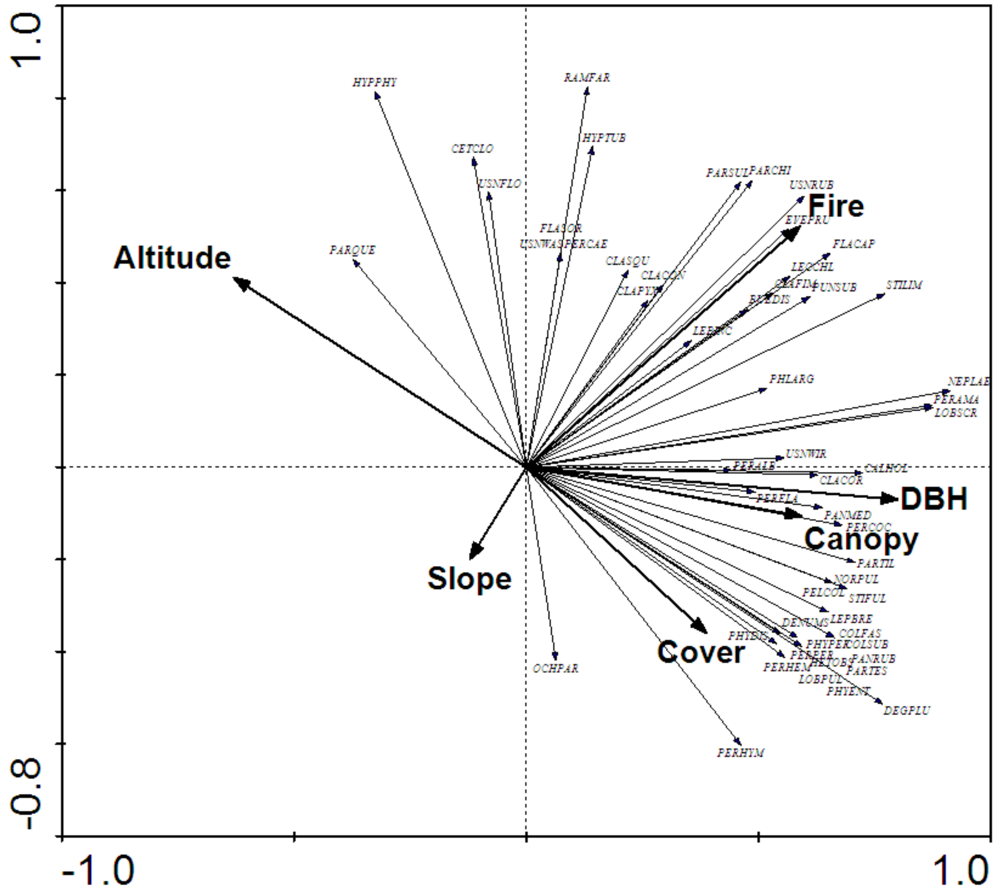

Figure 2. Ordination diagram of species along the first two principal components of the PCA. Environmental variables are passively projected into the ordination space. Abbreviations are according to table II (annex).

Principal Components Analysis (PCA) results (table 5a) show that the first two axes explain approximately 50% of the total variation. In the PCA ordination diagram (figure 2), it is possible to detect a relation between "old-growth" associated species and DBH, tree cover and canopy density. Ubiquitous species such as *Flavoparmelia caperata and Parmotrema chinense* appear to be more associated with fire and present a similar behavior in their post-fire response, increasing in frequency with time since fire, independently of forest structure. The length of the lines of the explanatory variables indicates a strong positive correlation between axis 1 and forest structure related variables (especially canopy density and DBH). Time since fire (Fire) is positively correlated with both axis 1 and axis 2 (see also table 5b). Results from Redundancy Analysis (RDA) in which axis 1 is restricted to be a linear combination of fire is presented in figure 3, in terms of species richness.

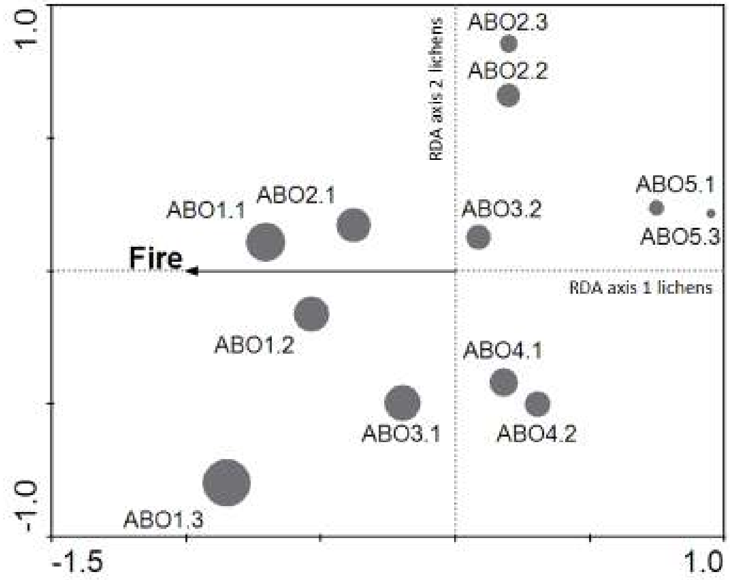

Figure 3. Ordination diagram of sample units along axis 1 and 2 of RDA. The size of the circles represents species richness. ABO5: burned 3 years ago, ABO4: burned 8 years ago, ABO3: burned 13 years ago, ABO2: burned 18 years ago, ABO1: unburned forests.

Species diversity and species richness present the same behavior along the post-fire gradient. ABO1 (3 years since last fire) is positively correlated with fire (axis1) and ABO5 (unburned forest) negatively correlated. The distribution of all other sites along the first ordination axis is fire related.

Ecological traits

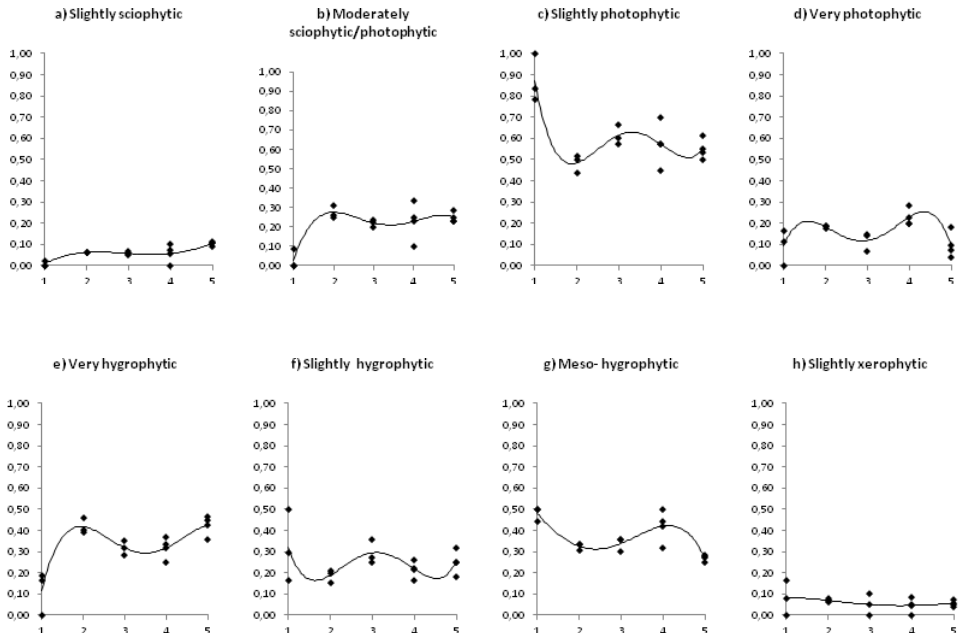

Figure 4. Effect of fire on relative richness of classes defined by photophytic index (a, b, c and d) and hygrophytic index (e, f, g and h). See table 1 for details of classes. Fire gradient classes, 1: burned 3 years ago, 2: burned 8 years ago, 3 burned 13 years ago, 4: burned 18 years ago, 5: unburned forests.

Considering hygrophylly (figure 4), only the very hygrophytic and meso-hygrophytic species showed a significant overall effect of fire on their relative richness. Very hygrophytic species were the only species exhibiting a clear positive trend along the gradient (figure 4e; table 4). Interestingly, their relative richness decreased from 8-years ago to 13-years since last fire.

A different pattern was revealed looking at the different classes of photophylly (figure 4), where only slightly sciophytic and slightly photophytic showed significant differences along the gradient (table 4). Relative richness of slightly sciophytic species was significantly higher while slightly photophytic species was significantly lower in unburned forests. Furthermore, relative richness of these trait classes changed linearly from recently burned to unburned forests (table 4; figure 4a-d).

Vegetative traits