Structure, regeneration and functional dynamics of fragmented Mediterranean forests

Sara Maltez Mouro

A thesis submitted for the degree of Doctor of Ecology

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A thesis supervised by Professor Helena Freitas and Doctor Teodoro Marañón

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Dedication

I dedicate this thesis to:

my father, who has always given me incentive to pursue new knowledge on everything;

my mother, who has always pushed me to work when motivation was scarce;

my sister, who has always supported my decisions, but first making me think about the disadvantages.
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Acknowledgements

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(http://alojamientos.us.es/grnm210/index.htm) was very motivating, and the support, kindness and hospitality of each member of those groups was also a great help.

Finally, I must say that this thesis has been an incredible experience, which would never have been possible without the direct or indirect support and encouragement of my family.
Abstract

Forest ecosystems provide goods and services with inestimable value. In particular, well-preserved forests are extremely important as benchmarks for monitoring the effects of human-caused changes of the environment, and to measure the sustainability and ecological importance of disturbed ecosystems. However, the world is changing fast towards a less forested planet, because of the destruction, disturbance and change of primitive forests, which make them more rare and vulnerable to an equilibrium rupture and ecosystem disruption. At the south of Portugal, only small, rare and disperse patches of natural forests remain nowadays, due to the particularly long human activity typical of all the Mediterranean Basin.

The main aim of this thesis was to investigate the functional structure and dynamics of persisting natural forests, which is an essential knowledge to assure their long-term persistence. The study site (37º40’N 8º43’W) is a mixed-oak Mediterranean forest, located at the Natural Park of Sudoeste Alentejano e Costa Vicentina. It was chosen for being a rare, natural and extremely well-preserved Mediterranean forest, and its dominant tree species are Quercus suber (cork oak, evergreen) and Q. faginea (“carvalho-cerquinho”, deciduous). The following questions and hypothesis were unfolded:

a) Besides the direct influence of topography over species composition and abundance, do the overstory species or the community structure influence the floristic-environmental variation? (Ecosystem engineering hypothesis);

b) Is the spatial pattern of successfully established recruits of woody species different from random? (Hypothesis of non-random distribution of regenerating plants);

b) Is the spatial pattern of successfully established recruits of woody species different from random? (Hypothesis of non-random distribution of regenerating plants);

c) Are there positive and/or negative associations between recruits of different species? (Intra-species bivariate and multivariate spatial patterns);

d) Are the spatial patterns of recruits positively or negatively associated to spatial variations of micro-environmental variables or to the occurrence of any species in the overstorey canopy? (Regeneration niches);

e) How does the topographic position or the canopy species composition influence the process of litter decomposition? (Litter decomposition rate);
f) Do the direct and indirect effects of the overstorey canopy cause opposite gradients in the availability of resources, in particular light and key soil nutrients? (Counteracting gradients).

To identify the main gradients in the studied forest, floristic, micro-environmental, edaphic and community structure variables were assessed, along three 50m long transects of 4x4m and 4m spaced plots (giving a total of 25 plots). The regeneration potential and spatial patterns of the forest, were studied after establishing two 32 x 10 m plots (one in the lower part of the forest slope, and another in the upper-slope). In each of these two plots, every single recruit of the four dominant woody species was mapped. A 0.25m radius area was considered around each, to measure the micro-environmental and community structure variables. To assess the in situ potential of litter decomposition, a litterbag experiment was performed along the 32m bottom border of each of those two plots.

A sequence of several complementary analyses was used:

i) The variation of soil chemical variables along the slope was analyzed by principal components analysis (PCA) on the correlation matrix;

ii) The floristic gradients and their relationship with edaphic factors, as well as the relationships between environmental variables and the species recruitment, were examined by stepwise canonical correspondence analysis (CCA);

iii) The floristic variance explained by non-spatially structured edaphic variables, was determined using partial CCA (pCCA);

iv) The spatial component of floristic variance not shared with edaphic variables, was obtained by another partial CCA;

v) The relationships between the environmental variables and the overstorey were determined using Student t-test and Spearman rank-correlation coefficients;

vi) The independency between the successful recruitment and the overstorey canopy species, was tested using the chi-square test;

vii) To investigate the recruitment spatial pattern at the community level, the modified Rippley’s K-function (L(r)) was used;
viii) The spatial pattern of each species (conspecific associations) was analysed using the **J-function**, while the pattern of each recruit species relative to the remaining species (interspecific multivariate analysis) was calculated, using the `type i to any type` **multitype J function**.

ix) To determine the litter decomposition rate, two models were fitted using **non-linear mixed effect models**;

x) The relationships between canopy density, litter accumulation and topsoil N and P levels in the understorey were analyzed by **correlation analyses**;

xi) To investigate if the overstorey canopy indirectly affects the topsoil N and P availability, throughout litter accumulation, the **path analysis by Shipley’s d-sep method** was used, testing both **separate and global causal models**.

Results showed that the studied Mediterranean natural forest is not homogeneous along the slope. The bottom-top gradients identified refer to the floristic, edaphic (i.e. soil fertility), community structure, and micro-environmental characteristics of the forest ecosystem. Besides, results also revealed that independently from the previously described top-bottom gradients, there was a close relationship between the dominant oak species and the edaphic variables, which suggests that the dominant oak species may act as ecosystem engineers, modulating light and edaphic resources from which the other plant species are dependent.

Regarding the regeneration processes, results showed a non-random distribution of some species of recruits and the existence of inter-species differences. The recruitment patterns were influenced by the overstory structure and composition; in particular, the recruits’ distributions and associations were explained by the recruits’ dependence on the mother trees and the light, litter, soil moisture and overstory canopy species. These results suggested that the overstory canopy have both direct and indirect effects on regeneration, and thus on the overall community structure and composition.

The litterbag experiment showed that the litter accumulated in the lower or upper forest communities had significantly different water content. However, the litter decomposition rate was the same (-0.6year\(^{-1}\)) in both extremes of the studied forest.
Finally, the detailed inspection of the gradients related to canopy density, litter accumulation and soil nutrients, revealed that they covariate. Besides, it was shown that the overstory canopy had counteracting effects over light and nutrients.

**KeyWords:** ecosystem engineers, gradients, litter decomposition, Mediterranean forest, *Quercus suber, Quercus faginea*, spatial patterns, variance partitioning.
Resumo

Os ecossistemas florestais proporcionam ao Homem bens e serviços de valor inestimável. O estudo das florestas naturais (ou em bom estado de conservação) em particular, é extremamente importante e serve de situação de referência para a monitorização dos efeitos da acção do homem no ambiente e para avaliar a sustentabilidade e valor ecológico dos ecosistemas perturbados. No entanto, são profundas e rápidas as alterações que sucedem à escala planetária no que respeita à área ocupada pelos ecossistemas florestais: a destruição, perturbação ou alteração das florestas primitivas torna-as mais raras e vulneráveis a uma ruptura do seu equilíbrio ecológico e à perda de todo o ecossistema. No sul de Portugal, actualmente apenas persistem áreas pequenas, raras e dispersas de floresta natural, devido à imensa história de actividade humana, típica aliás de toda a Bacia Mediterrânica.

O grande objectivo desta tese foi investigar a estrutura funcional e a dinâmica das florestas naturais, sendo esse um conhecimento essencial para garantir a sua longa existência. A área de estudo localiza-se no Parque Natural do Sudoeste Alentejano e Costa Vicentina e é uma floresta mista de carvalhos que ocupa uma vertente inclinada (37º40’N 8º43’W). Foi escolhida por ser uma floresta Mediterrânica natural, rara mas extremamente bem conservada, onde as espécies arbóreas dominantes são Quercus suber (sobreiro, sempre-verde) e Q. faginea (carvalho-cerquinho, decídua). Como ponto de partida, foram consideradas as seguintes questões e hipóteses:

a) Para além da influência directa da topografia na composição específica e na abundância das espécies, será que as espécies da canópia ou a estrutura da comunidade influenciam a heterogeneidade florístico-ambiental? (Hipótese de engenharia do ecossistema);

b) O padrão espacial das plântulas de espécies lenhosas que se estabeleceram com sucesso é não-aleatório? (Hipótese da “não-aleatoriedade” na distribuição das plântulas);

c) Existem associações positivas e/ou negativas entre as plântulas de diferentes espécies? (Padrões espaciais intra-específicos, bivariados e multivariados);
d) Os padrões espaciais das plântulas estão positiva ou negativamente associados às variações espaciais das variáveis micro-ambientais, ou à ocorrência de determinadas espécies ao nível da canópia? (Nichos de regeneração);

e) Como é que a posição topográfica ou as espécies constituintes da canópia influenciam o processo de decomposição da folhada? (Taxa de decomposição da folhada);

f) Os efeitos directos e indirectos da canópia causam gradientes opostos relativos à disponibilidade de recursos, em particular luz e nutrientes? (Gradientes opostos).

Para identificar os principais gradientes no bosque estudado, foram consideradas variáveis florísticas, micro-ambientais, edáficas, e variáveis relativas à estrutura da comunidade, ao longo de três transectos com 50m de comprimento, com áreas amostrais de 4x4m e espaçadas 4m ente si (i.e. um total de 25 áreas de amostragem). O potencial de regeneração e os padrões espaciais, foram estudados estabelecendo duas áreas de amostragem de 32x10 m (uma na zona mais baixa da vertente ocupada pelo bosque, a outra na zona alta). Em cada uma destas áreas, foi registada a localização de cada plântula das quatro espécies lenhosas dominantes. Em cada plântula foi centrado um círculo com 0.25m de raio, para medição das variáveis micro-ambientais e relativas à estrutura da comunidade. Para avaliar o potencial de decomposição da folhada in situ, foi feita uma experiência com “litterbags”, ao longo dos 32m correspondentes ao limite inferior de cada uma das grandes áreas de amostragem.

A análise dos dados teve como base a seguinte sequência de várias análises complementares:

i) A variância das variáveis relativas à composição química do solo, ao longo da vertente, foi determinada pela análise dos componentes principais (ACP) sobre a matriz de correlação;

ii) Os gradientes florísticos e a sua relação com os factores edáficos, bem como as relações entre as variáveis ambientais e as plântulas a regenerar, foram examinados por análise canónica de correspondência passo-a-passo (ACC);

iii) A variância florística explicada por variáveis edáficas sem estrutura especial, foi determinada por uma ACC parcial;
iv) Os componentes espaciais da variância florística independentes das variáveis edáficas, foram determinados por outra ACC parcial;

v) As relações entre as variáveis ambientais e a canópia foram avaliadas utilizando testes t-Student e coeficientes de correlações de Spearman;

vi) A independência entre plântulas bem sucedidas (a regenerar) e as espécies presentes na canópia, foram testadas pelo teste do chi-quadrado;

vii) Para investigar os padrões espaciais de regeneração ao nível da comunidade, foi utilizada a função Ripley’s K modificada (L(r));

viii) O padrão espacial de cada espécie (associações conspecíficas) foi analisado utilizando a função J, enquanto o padrão de cada espécie de plântulas realetivamente às outras espécies (análise interspecífica multivariada) foi calculada pela função J multivariada “type i to any type”;

ix) Para determinar a taxa de decomposição da folhada, foram ajustados modelos mistos não lineares;

x) As relações entre a densidade da canópia, a acumulação de folhada, e os níveis de N e P no solo, foram avaliadas por análises de correlação;

xi) Para investigar se a canópia tem efeitos indirectos na disponibilidade de N e P, via acumulação de folhada, recorreu-se à “path analysis” pelo método d-sep de Shipley, testando quer modelos separados quer modelos globais.

Os resultados mostraram que a floresta Mediterrânia natural estudada não é homogénea ao longo da vertente. Foram identificados gradientes florísticos, edáficos (i.e. fertilidade do solo), estruturais, e micro-ambientais. Os resultados mostraram ainda que, independentemente desses gradientes ao longo da vertente, existia uma estreita relação entre a espécie de carvalho dominante e as variáveis edáficas, o que sugere que essa espécie possa actuar como engenheira do ecossistema, modulando a luz e os nutrientes de que dependem as outras espécies.

Relativamente aos processos de regeneração, os resultados mostraram uma não-aleatoriedade na distribuição de algumas das espécies de plântulas, e a existência de diferenças inter-específicas. Os padrões de regeneração foram influenciados pela estrutura e composição específica da canópia; em particular, a distribuição e as
associações das plântulas foram explicadas pela sua dependência relativamente à árvore-mãe, e pela luz, folhada, humidade do solo, e composição específica da canópia. Estes resultados sugerem que a canópia tem tanto efeitos directos como indirectos na regeneração, influenciando portanto toda a estrutura e composição da comunidade.

A experiência de decomposição mostrou que a folhada acumulada na zona alta ou na zona baixa da vertente, é significativamente diferente no que respeita ao seu conteúdo hídrico. Por outro lado, as taxas de composição da folhadas foram idênticas (-0.6 year\(^{-1}\)) nos dois extremos do bosque estudado.

Finalmente, verificou-se que os gradientes de densidade da canópia, acumulação de folhada, e nutrientes no solo, covariam. Além disso, mostrou-se que a canópia tem efeitos opostos ao nível da luz e dos nutrimentos.

**Palavras Chave:** decomposição, espécies engenheiras, floresta Mediterrânica, gradientes, *Quercus suber, Quercus faginea*, padrões espaciais, partição da variância.
List of papers, oral presentations and posters

Papers


Oral presentations

• Maltez-Mouro S., L.V. García, T. Marañón, and H. Freitas. Understory floristic heterogeneity within a Mediterranean oak forest: how much is explained by
edaphic, spatial and overstory components? 9º Encontro Nacional de Ecologia, SPECO (Universidade de Coimbra, 14 a 16 de Outubro de 2004).


- Maltez-Mouro S., T. Marañón, and H. Freitas. Woody plant regeneration and overstory heterogeneity within a mixed oak Mediterranean forest (Portugal). 90th ESA Annual Meeting & IX INTECOL Congress (Montreal, Canada, 7th to 12th August 2005)


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Chapter 3.2.

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I. Introduction
I. Introduction

1.1. The forest ecosystem

Forests are ecological complex systems functioning as a whole. They follow the ecosystem definition, being integrated and dynamic, and embracing many interactions between live organisms – including humans – and non-live components. Forests represent the largest and most ecologically complex systems; they contain a wide assortment of trees, plants, mammals, reptiles, amphibians, invertebrates, insects and micro-organisms that vary depending on the zone's climates.

Forest ecosystems are extremely important for the biological diversity, goods and services they embrace and provide to modern societies. For example, forest ecosystems provide goods with commercial value, such as timber, fuel, charcoal and medicinal plants; they also provide services with huge and inestimable value, because of their determinant role in the carbon cycle, hydrological cycle (including water quality), global and local climate, soil protection against erosion, biological and genetic diversity, and air purification. Besides, it has long been recognized their traditional, spiritual, psychological, recreation, tourist and landscape values. Well-preserved forests are particularly important as benchmarks for monitoring the effects of forest management practices, air pollution, and other human-caused changes of the environment, and to measure the sustainability and ecological importance of disturbed ecosystems.

The complete destruction or the disturbance and change of primitive forests, deserve the world’s concern and special attention, because disturbed forests become more vulnerable to stress, invasive species, patogenic insects and micro-organisms, and climatic changes, which ultimately cause the ecosystem disruption. Nowadays, forests occupy around 30% of the non-ice area of the Earth, but this percentage is changing fast towards a much less forested planet. Some international, national, and regional decisions and actions have been recognized as urgent in order to preserve forest ecosystems.

1.2. Mediterranean forests today

Mediterranean forests are characterized by hot-dry summers and mild, rainy winters, with nearly all rainfall occurring in the winter and spring rainy seasons. They exist in five
Mediterranean climate zones, on the west coast of continents in mid-latitudes (30º – 45º N or S) and occupy less than 5% of the Earth's surface (Figure 1.1). In the Mediterranean zones, along with Mediterranean forests other vegetation types are present such as savannas, shrublands and grasslands. These different types of vegetation are commonly interleaved with one another in complex patterns created by variations in soils, topography, fire history, and exposure to wind and sun, usually known as “mosaic” landscape.

![Map of Mediterranean-climate regions of the world: the Mediterranean Basin, California, Central Chile, Southwest Australia, the Cape Province of South Africa, and southeastern tip of Vancouver Island, Canada (adopted from DiCastri et al. 1981)](image)

The Mediterranean Basin has a particularly long influence from human activity. The forests have been cut and used to satisfy human immediate needs, thus flatlands have been mostly deforested and transformed to croplands and grasslands, and most of the remnant natural forests are restricted to mountains and rough lands (Thirgood 1981).

Forests have been directly destroyed by human activities that also indirectly damage their functional and ecological integrity. Fragmentation is one of the main injuries of the human activity, both at structural and landscape level. Although fragmentation has often been associated to an increase of species richness, that increment arises from the margin
effect, corresponding to the increase of generalistic species but a decrease in the number of species characteristic from non-fragmented forests.

Fire, both natural and human-caused, has played a determinant role in Mediterranean ecosystems. The hot, dry summers make much of the region prone to fires, and lightning-caused fires occur with some frequency. Historically, natives extensively used fire to clear brush and trees and promote the growth of grasses and herbaceous vegetation that supported game animals or that were directly more useful for humans. In the last few decades, human-caused fire has shown increasing intensities and rates of occurrence (Gómez et al. 2004) for no apparent reason, and it has been the major cause of forests degradation and loss in Mediterranean regions.

Along with the direct huge destruction of vegetation, fire has negative effects at both structural and functional levels, and it has an enormous impact at the landscape and aesthetic levels. It also has strong negative impacts on the whole ecosystem, namely on soil cycles (and erosion), hydrologic cycle, biogeochemical cycle, nutrients cycle, vegetation succession, habitats availability, biological diversity, biomass, regeneration, productivity, and shelters (Schulze et al. 2002, p.418; Arroyo et al. 2004, p.34; Marañón et al. 2004a, p.93). The huge amounts of gases and particles released to the atmosphere, along with the destruction of the carbon-sinks themselves, make fire responsible for the most concerning and recognized problems at the global scale: the ozone hole and the warming effect (e.g. Russel 1998).

1.3. The Portuguese forests

Nowadays, forest ecosystems occupy 38% of the Portuguese continental territory. Historically, they were first cut to obtain grazing areas and then for ship-building. After that, pinus forests (Pinus pinaster Aiton) were planted, explored for resin extraction and periodically clear-cut. More recently, many forests have suffered from active species selection, clear-cut or cut down, and the endogenous species have been replaced by exotic species such as Eucalyptus globulus Labill, used for paper industry. Besides, in the last few decades, fire has destroyed 120.000ha per year (in average) of the Portuguese forested areas (Dir. Nacional da Quercus 2003).
Roads construction and urbanization have also been major factors contributing to forests destruction and degradation.

At the south of Portugal, in the western part of the Mediterranean Basin, only small, rare and disperse patches of natural forests remain nowadays, and they persist only on steep slopes. Therefore, it is of increasing importance to understand the functional structure of these forest fragments and to assure their long-term persistence.

1.4. Structure and functioning

It has long been recognized that forest ecosystems are not static in time (e.g. Ljungman et al. 1999, Schulze et al. 2002, p.417). Usually, they are also not homogeneous in space, presenting abiotic, functional and structural heterogeneities, from micro to global scale. The abiotic heterogeneity has been the most studied one (e.g. Marañón et al. 2004a, Gómez-Aparicio 2005) and refers to the variability of resources and environmental factors such as light, water and temperature, which always influence the species presence and individual performance. The functional heterogeneity corresponds to the variability of a system property that affects ecological processes, and the structural heterogeneity is the variability of a system property that is measured without reference to any ecological effect (Marañón et al. 2004a).

It is essential to analyse and foresee the structure and functioning of the forests, if we want to forecast the vegetation dynamics at the landscape or at the community level, and to anticipate the risk of invasion by exotic species (Burke and Grime 1996). It has also been claimed the urgent need to investigate the vegetation dynamics at the smallest scale: small groups of trees play a determinant role at small scales, as far as their ecological niches have strong influence in the development of other species, but their role at that level is not detectable at larger scales (Carrer and Urbinati 2001).

1.5. Gradients and patterns

The forest species distribution is influenced by the spatial and temporal heterogeneity of resources. This heterogeneity and the forest conditions affect the performance of individual organisms and the differential response among tree species at the seedling stage, thus promoting the coexistence and maintenance of the forest biodiversity (George
The species spatial variability also arise from a combination of mechanisms such as density-dependent regulation of populations, competitive exclusion, regeneration dynamics, disturbances (e.g. fire), canopy gaps, etc. (e.g. Honnay et al. 2001, Takahashi et al. 2001).

The forests’ overstory-understory relationships are strongly related to the forest dynamics and spatial heterogeneity (George and Bazzaz 1999). It has been recognized that overstory trees modify the environment underneath and therefore influence species composition and plant growth in the understorey (including seeds and seedlings); in turn, the differential success among plants and species in the understorey (including regeneration success), affects the future overstory composition (Foré et al. 1997).

On forested steep slopes, topography is an extremely relevant ecological factor that determines species spatial heterogeneity. From top to bottom of any slope, changes in the physical environment are associated with a complex environmental gradient, including changes in microclimate, water drainage and accumulation, and transport and accumulation of soil minerals. All these factors directly or indirectly affect the spatial patterns of plant species distribution and abundance, and the topography-related variation in forest ecosystems have been documented in many studies (e.g. Tokuchi et al. 1999, Clark et al. 1998, Hanba et al. 2000, Ojeda et al. 1996, 2000). However, variation in species abundance and forest structure along with topographic gradients, are not uniform. On one hand, different mechanisms may affect the vegetation differentiation along topographic gradients (e.g. Hirayama & Sakimoto 2003); on the other hand, many sources of floristic variation are independent of topography (Hirayama & Sakimoto 2003).

Spatial patterns constitute a kind of ‘ecological fingerprint’ that identify the essential processes and structures of a system (Goreaud and Pélissier 2003). They also relate to specific mechanisms and play a key role in the ecosystem dynamics. At the species level, spatial patterns indicate the substituting pattern and the stable coexistence of the species (Takahashi et al. 2001). At the community level, spatial pattern analysis is an explicitly inductive tool (Silvertown and Wilson 1994), which is very useful to test for the existence of structure in the community, and to support the interpretation of processes that have produced the observed patterns (Moeur 1993, Miller et al 2002). Therefore, the
distribution patterns and species associations or repulsions, reflect the ecology of the forest ecosystem and are an essential knowledge for management, recovery or sustainability needs (Miller et al. 2002).

1.6. Engineering species

One interesting mechanism to explain the vegetation heterogeneity is based on the recently described and recognized concept of trees as “physical ecosystem engineers”. It states that the mere presence of physical structures (such as trees), their continual growth and replacement, and their persistence over long periods of time, may lead to the modulation of resources, from which other plant (and animal) species are dependent (Jones et al. 1997). In fact, it has long been recognized that the local distribution and abundance of tree species have important consequences for both community structure and ecosystem-level processes (Tilman 1988, Schlesinger et al. 1990).

Single trees affect the light environment (Gómez et al. 2004), the litter abundance on the forest floor, and the physical and chemical properties of the soil. Therefore, the spatial distribution of tree canopies along forested slopes takes active part in the control and modulation of abiotic resources, and leads to heterogeneous physical environments that are perceived by seeds and seedlings.

1.7. Regeneration niches

Differentiation mechanisms occur mainly at the seeding phase. Therefore, the recognition of abiotic and biotic factors that correspond to the regeneration niches of different species is extremely important to understand, modulate and foresee the forest dynamics (Marañón et al. 2004a).

The first definition of regeneration niche was given by Peter Grubb (1977 in Marañón et al. 2004a) to explain the coexistence of individuals that do not differ in basic use of resources but have different needs at the regeneration phase. According to a revised definition of niche concept (Chase and Leibold 2003), both the species' requirements and the species' impacts on the environmental conditions must be accomplished. The recognized components of a regeneration niche include the hydrologic response, light response, resistance to extreme climatic conditions (e.g. occasional snow, frost, and very
high temperatures), soil chemical composition, levels and type of predation, adult trees or shrubs, bacterial and fungal livings in soil, fire resistance, etc.

The spatial patterns of successfully established tree seedlings depend on a chain of regeneration processes, including dispersal patterns, germination and emergence responses, and seedling growth and survival. Besides, it has been recognized that different species have different multidimensional niches. This, combined with the spatial and temporal heterogeneity of the forest systems, favours the coexistence and maintenance of the forests diversity (Marañón et al. 2004a).

1.8. Litter decomposition: an essential process

Litter decomposition is an essential process in the functioning of forest ecosystems, through which the fixed carbon that has been incorporated into above and belowground biomass, returns to the atmosphere (as CO₂) and incorporates into the soil (i.e. converts into stable soil organic matter) (Schulze et al. 2002). Besides, the release of nutrients from decomposing litter is an important internal pathway for nutrient fluxes in forested ecosystems, with nutrients becoming available for plant uptake and being not lost from the system. Litter is released from adult trees, which have big root systems and thus capture nutrients from mineral deep soil and release them to superficial soil through litter; these nutrients at the superficial soil, favour the growth and survival of regenerating individuals, which have short and superficial roots.

It has been described that the process of litter decomposition is controlled by biotic and abiotic factors, and by the litter quality and quantity (e.g. Murphy et al. 1998). Besides, the single-species dynamics do not correspond to the decomposition patterns of the ecosystem (Gartner and Cardon 2004). Therefore, the decomposition experiments must start from the real litter mixture found in the ecosystem (collecting litter from the ground and using a representative proportion of each species), and must put back litter in the field to decompose in situ. That is the case of the litterbag experiment performed in this Thesis: it takes into account all the biotic, abiotic, and litter characteristics of the ecosystem, gives and easily measured rate of litter decomposition, and allows to compare communities relative to one another.
1.9. Counteracting gradients

The relationships between the litter decomposition, the overstorey structure and canopy species, and the regeneration processes (including survival and growth), is essential to understand the whole ecosystem functioning and dynamics (Zamora et al. 2004, Herrera et al. 1994). However, the overstorey structure and composition have direct effects on light availability in the understorey that are not easy to distinguish from their indirect effects on the growth and survival of understorey plants. Those indirect effects are mediated by litter accumulation, which determines the organic debris and the input of mineral nutrients into the forest floor (e.g. Ross et al. 1986, Figueroa-Rangel and Olivera-Vargas 2000, Gallardo 2003) – i.e. relate to soil nutrients availability. From the occurrence of both direct and indirect effects of the overstorey, we may expect that some counteracting gradients exist between different key resources in the understorey, such as light and soil nutrients. The evaluation of the potential impact from the overstorey over the understorey community, may explain some essential regeneration processes and the whole forest dynamics (Rossi and Villagra 2003, Broncano et al. 2004, Puerta-Piñero et al. 2006).

1.10. Layout of the thesis

According to the most general definitions of Mediterranean climate, nowadays the Iberian Peninsula is the larger contiguous area under that climate all over the world (Gómez et al. 2004). However, natural and mature Mediterranean forests of the Southwest Portugal persist only in small, rare and disperse patches, and they must be studied before they suffer any major disturbance.

The main objective of this thesis is to improve the knowledge on the functional structure and regeneration capacity of remaining natural Mediterranean forests. We believe that it ultimately contributes to delineate management actions needed for their long-term persistence.

The field-work of this thesis focused on one mixed-oak natural forest (with Quercus faginea Lam. and Q. suber L.) located on a steep slope at Southwest Portugal. It included observational data collection and one experimental approach. The main objectives came to grips with the three papers presented in Chapter III, two of them published in
international scientific journals quoted at the Science Citation Index (SIC), and the other published in an international electronic journal issued by the European Ecological Federation in cooperation with the Oikos Editorial Office. The objectives of the experimental approach were embraced in Chapter IV, which presents data not yet published.

The structure, general aims and objectives of the thesis can therefore be summarized as:

1) Observational study (Chapter III, which includes two published papers).
   a) To study in deep the structural and floristic variation of the studied forest (Chapter 3.1).
      i) To describe the floristic variation along the slope;
      ii) To describe the edaphic, topographic, and community structure (all together named environmental variables) variations along the slope;
      iii) To relate the floristic gradients with the environmental gradients; partialling out the spatial components (i.e. to determine the non-spatially structured floristic-edaphic variance) and the edaphic components (i.e. to determine the spatially structured floristic variance);
      iv) To identify the relationships between dominant oak species and edaphic variables independently from the bottom-top gradients.
   b) To investigate the spatial patterns of recruits of the main woody species (Chapter 3.2).
      i) To investigate the recruitment spatial distributions and associations (i.e. pattern) at the community level;
      ii) To characterize the microsite environment of recruits and relate the environmental variables with the recruitment of the studied species;
      iii) To assess the role of the canopy structure and composition on the recruits spatial pattern;
      iv) To infer the mechanisms affecting the regeneration of the studied main woody species in particular, and Mediterranean forests in general.

2) Experimental study (Chapter IV, which includes data not yet published).
   i) To determine the litter decomposition rate at contrasting slope positions;
ii) To compare the litter decomposition rate at distinct communities, one dominated by *Q. faginea* and the other dominated by *Q. suber*;

iii) To compare the litter decomposition rate at the studied forest with the ones described for other ecosystems;

iv) To evaluate how the topography and tree species composition relate to the litter decomposition rate.

3) Joint study comparing the field data of this thesis with data from a mixed-oak forest at the South of Spain (Chapter V, which includes one published paper).

i) To analyse the relationships between woody canopy density, litter accumulation, and topsoil N and P levels in the understorey of those two forests;

ii) To evaluate if the direct and indirect effects of the canopy overstorey cause opposite gradients in the availability of resources (i.e. light and key soil nutrients).

Published papers were included in this thesis without changing the journals demands, and therefore the Chapters III and V have different shapes.

The whole thesis follows the nomenclature from Franco (1971, 1984).

1.11. References


II. Material and methods
II. Material and methods

2.1. Study area

The study area is a natural forest located at southwest of Portugal, in the Sudoeste Alentejano e Costa Vicentina Natural Park (Figure 2.1), near the Mira river and the Casa Branca settlement (37°40’N 8°43’W).

Figure 2.1 – Map showing the limits of the Sudoeste Alentejano e Costa Vicentina Natural Park (blue line) and the location of the study site (dark green ellipse).
After a detailed survey of the forest patches persisting in that region of Portugal, our forest site was chosen for being one of the largest and best preserved natural fragments. No records of fire or logging are known for this forest since at least 1956, and management is limited only to the periodic (at approximately 9 years interval) removal of bark from the largest cork oak (*Q. suber*) trees, for cork production. The studied forest is 400 x 70 m large and situated in a north-faced 32° slope (Figure 2.2). It is a mixed oak forest, where the dominating tree species are *Quercus suber* (cork oak, evergreen) and *Q. faginea* (“carvalho-cerquinho”, deciduous).

Figure 2.2 – Satellite image of the studied forest patch. Image from Google Earth 3.1.0621.0(beta).

The climate is of Mediterranean-type, with mild humid winters and warm dry summers. Mean annual precipitation is ca. 600mm, with only 10% occurring between May and September, and mean annual temperature is ca. 15°C, with mean maximum and minimum annual temperatures of ca. 29°C and 6°C, respectively. The main bedrock is greywacke,
and the soil is moderately acidic (mean pH of 5.0) and with low fertility (especially in N and P).

### 2.2. Sampling methods

To identify the main gradients in the studied forest patch (Chapter 3.1), floristic, microenvironmental, edaphic and community structure variables were assessed along the bottom-top hillside. Data was collected at linear sequences (transects) of 4 m spaced, 4 x 4 m plots (Figure 2.3). After excluding edges, shrubland and path areas at the hilltop, each transect was approximately 50 m long. Three parallel to each other transects were traced, giving a total of 25 (4 x 4m) plots.

![Figure 2.3 – Approximate location of the 25 plots aligned at three bottom-top transects, in the studied forest, at a north-faced slope.](image)

The regeneration potential, the regeneration ecology, and the regeneration spatial patterns of the selected forest community (Chapter 3.2) were studied after establishing two plots of 32 x 10 m: one in the lower part of the forest slope, which was dominated by *Quercus faginea*, and another in the upper-slope, dominated by *Q. suber* (Figure 2.4).

![Figure 2.4 – Approximate location of the two plots (32 x10m each) established to study the forest regeneration, one more close to the bottom and the other located upper in the slope.](image)
The litter decomposition rate (Chapter IV) was measured along the 32m bottom line of each of the two 32m long plots referred above, following the method described by Quested and Garnier (2003).

### 2.2.1. Floristic data

The floristic data included the number of individuals of each woody species (excluding lianas), which was measured in each 4x4 m plot of the three transects. For individuals more than 1.30 m tall, the height, trunk diameter at breast height (dbh) and the number of trunks at the base (in the case of plants branching from the base) were measured.

### 2.2.2. Regeneration data

In the two 32 x 10m plots established to study regeneration, every single recruit of the five dominant woody species (*A. unedo, Q. faginea, Q. suber* and *V. tinus*) measuring 10 to 130cm height, was sampled. Considering circular areas of 0.25 m radius centered on each recruit, the following variables were recorded: height; diameter at 10 cm height; number of trunks at the base; distance to the nearest adult of the same species; distance to the nearest adult of a different species; name of the nearest different species; the overlapping canopy species and height; spatial position inside the plot (X, Y coordinated relative to the plots’ border); and the micro-environmental variables referred in the next sub-section.

The same variables, except those directly related to the recruits characteristics or distances to adult neighbors, were also recorded at forty random locations inside each plot.

### 2.2.3. Edaphic, micro-environmental, and community structure data

Both at the plots aligned in bottom-top transects and at the nearby of each recruit, the micro-environmental variables measured were: ground-cover percentage of perennial herbaceous vegetation, moss and leaf litter, by visual direct estimation at increments of 5%; litter depth, with a ruler; maximum canopy height, at class intervals of <2m, 2-4m, 4-6m and >6m; canopy density, using a spherical densiometer positioned 1m above the
ground (Figure 2.5); soil humidity, using a Time Domain Reflectometry (TDR) probe; and slope, using a clinometer.

Figure 2.5 – Spherical densimeter used in the field work. This device is composed by a concave mirror with 37 grid intersections forming 24 squares; the user assumes four equi-spaced dots in each square of the grid and systematically counts the dots equivalent to quarter-square openings. Multiplying the total count by 1.04, the percent of overhead area not occupied by canopy is calculated. The difference between this and 100 is an estimation of overstory density in percentage. Measurements were done after leveling this device at 1m or 50cm above ground, in the community study along the transects and regeneration study, respectively; total counts considered were the average value between counts made facing the four main cardinal points.

The topographic position (X, Y and Z coordinates) of the plots aligned in bottom-top transects, was measured using an electronic distance measurer (Figure 2.6; Box 1 for details).

Figure 2.6 – Measurement of the coordinates position of each plot aligned in three bottom-top transects, using an electronic distance measurer (EDM, Sokkia SET500). a, the tripod with the EDM device, on the opposite south-faced slope; b, the target, placed at least at two opposite corners of each of the 25 plots on the north-faced forest slope.
Soil chemical parameters were measured after sampling superficial soil (0-10 cm depth) with a corer (2.54 cm diameter), in each of the 25 plots aligned in transects. Six soil samples were randomly taken inside each plot, which were then mixed to have one soil sample per plot. Soil samples were dried, crushed and then passed through a 2 mm sieve. Finally, they were analysed for 19 chemical parameters: soil moisture (measured by gravimetric method), pH (in a 1:2.5 suspension with KCl 1M), organic C (by elemental analysis), organic matter (calculated from organic C multiplied by 1.724), total N (by Kjeldhal method), C/N ratio, exchangeable Ca, Mg, K and Na (by extraction with ammonium-acetate 1M pH 7.0), exchangeable acidity (EA, extracted with KCl 1M), effective cationic exchange capacity (CECe, calculated as the sum of the exchangeable cations), available P (by Olsen method), available Zn, Cu, Mn and Fe (by Lakanen method), B (extraction with hot water), and mineral N content (NH$_4^+$ and NO$_3^-$ extracted with KCl 1M 1:10). All the analysis followed the methods prescribed in Sparks (1996).

**Box 1**

The electronic distance measurer device (EDM, Sokkia SET500) measures the distance from the instrument to its target after sending out an infrared beam that is reflected back to the unit, and using timing measurements to calculate the distance traveled by the beam. A reflecting prism is normally used as the target, because the EDM requires that the target be highly reflective. The reflecting prism is a cylindrical device normally screwed into a bracket on the top of a pole; the pointed tip of the pole is placed on the points to be surveyed.

The device is mounted on a tripod and leveled before use, while the prism is mounted on a pole of known height. The device calculates the position of the prism, not the point to be surveyed, but since the prism is directly above the tip, the height of the pole is automatically subtracted to determine the location of the point. Therefore, the pole must be held upright, and a bubble level is attached to check that.

When the instrument is set up, its angle must be set considering the angle from north (i.e. zero degrees corresponds to the situation when it is actually pointing north).

In the field work of the thesis, the precise location of the EDM device (Figure 2.6a) was determined from 225 measurements with a Global Positioning System (GPS), so that the actual positions (northing, easting and elevation) of surveyed points could be calculated in absolute terms. The target was placed at least at two corners of each plot, for posterior calculation of
2.2.4. Litter decomposition experiment

The mass loss of litter placed in “litter-bags” was measured, to access the *in situ* “decomposability” of litter, i.e. its potential to decompose under its native field conditions.

A mesh was used to make ‘pockets’ with one open side (Figure 2.7a), which were filled with litter before the final seam along the top was sewn (Figure 2.7b).

The selected litter was dried at 20°C for 4 days. After that portions with 2g (+/- 0.1g) were put inside each litterbag, and finally litterbags were placed back in the field surface. Three litterbag harvests were made at six months intervals, i.e. the first harvest six months after placing litterbags in the field, the other after one year, and the last harvest after one year and a half. In each harvest, litterbags from eight positions were collected in each plot, thus a total of 48 litterbags (eight per plot and per harvest) were used.

The harvested material was oven dried 60 °C for two days, and weighed. To accomplish the actual mass loss due to the over-drying method, four litter portions (i.e. 2g of litter collected twice in each plot) were oven-dried and weighted without being placed back in the field.
2.3. Analysis of data

2.3.1. Floristic, edaphic, micro-environmental, and community structure gradients

Since many of the studied variables were highly skewed, transformations were applied prior analysis, as shown in Table 2.1.

Table 2.1 – List of measured variables for studying the edaphic, micro-environmental, and community structure gradients. Units and applied transformations are shown. See text for details and abbreviations.

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<td>-</td>
<td>-</td>
<td>P</td>
<td>mg kg(^{-1})</td>
<td>log</td>
<td>Slope</td>
<td>degrees</td>
<td>-</td>
</tr>
<tr>
<td>OM</td>
<td>g kg(^{-1})</td>
<td>log</td>
<td>Zn</td>
<td>mg kg(^{-1})</td>
<td>log</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>g kg(^{-1})</td>
<td>power (4)</td>
<td>Cu</td>
<td>mg kg(^{-1})</td>
<td>log</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/N</td>
<td>-</td>
<td>log</td>
<td>Fe</td>
<td>mg kg(^{-1})</td>
<td>log</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>cmol kg(^{-1})</td>
<td>log</td>
<td>B</td>
<td>mg kg(^{-1})</td>
<td>log</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>cmol kg(^{-1})</td>
<td>log</td>
<td>NH(_4^+)</td>
<td>mg N kg(^{-1})</td>
<td>log</td>
<td>Max. canopy height</td>
<td>m</td>
<td>-</td>
</tr>
<tr>
<td>K</td>
<td>cmol kg(^{-1})</td>
<td>log</td>
<td>NO(_3^-)</td>
<td>mg N kg(^{-1})</td>
<td>log (x+0.1)</td>
<td>Herbaceous cover</td>
<td>%</td>
<td>sqrt</td>
</tr>
<tr>
<td>Na</td>
<td>cmol kg(^{-1})</td>
<td>log</td>
<td>Litter</td>
<td>%</td>
<td>-</td>
<td>Moss ground-cover</td>
<td>%</td>
<td>log</td>
</tr>
<tr>
<td>EA</td>
<td>cmol kg(^{-1})</td>
<td>log</td>
<td>Litter depth</td>
<td>cm</td>
<td>-</td>
<td>Species richness</td>
<td>#spp/plot</td>
<td>-</td>
</tr>
</tbody>
</table>

Through a sequence of several complementary analyses, edaphic and floristic variations along the slope, their relationships, their relations with the micro-environmental and community structure variables, and the statistical significance of those relationships, were examined. Table 2.2 shows the method used, the input data matrixes and the output type of each step.

The variation of soil chemical variables along the slope was analyzed by principal components analysis (PCA) on the correlation matrix. The number of meaningful components was determined by the broken-stick method (Jackson 1993; King and Jackson 1999). Factor loadings exceeding 0.5 were used to interpret PCA components, as well as spearman rank-correlation coefficients between the extracted significant principal components and the topographic and community structure variables.
The variance partitioning, i.e. the amount of variance explained in each of the next steps, was calculated as percentage of total floristic variance. This was given by the sum of all eigenvalues in the correspondence analysis (CA) of the species abundance matrix (Borcard et al. 1992).

Table 2.2 – Summary of the analysis used to study the floristic, edaphic, micro-environmental, and community structure gradients. For each analysis (rows), the first column shows the commonly used name; the second column shows the input data matrix; the third column shows the data for which the first matrix is constrained, or the second data matrix used (for the two last rows); the fourth column shows the covariables used when some factors were partialed out; the last column shows the type of the output reached.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>First data matrix</th>
<th>Constrained by / Second data matrix</th>
<th>Covariables</th>
<th>Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCA</td>
<td>edaphic variables</td>
<td>-</td>
<td>-</td>
<td>significant principal components</td>
</tr>
<tr>
<td>CA</td>
<td>woody species</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CCA</td>
<td>woody species</td>
<td>edaphic data</td>
<td>-</td>
<td>edaphic predictors</td>
</tr>
<tr>
<td>CCA</td>
<td>woody species</td>
<td>spatial data</td>
<td>-</td>
<td>spatial predictors</td>
</tr>
<tr>
<td>pCCA</td>
<td>woody species</td>
<td>edaphic data</td>
<td>best spatial predictors</td>
<td>non-spatially structured edaphic predictors of floristic variance</td>
</tr>
<tr>
<td>pCCA</td>
<td>woody species</td>
<td>best spatial predictors</td>
<td>edaphic, topographic, and community</td>
<td>spatial predictors of floristic variance not shared with edaphic variables</td>
</tr>
<tr>
<td>spearman r</td>
<td>overstory species</td>
<td>total basal area, and mean values of the structure variables</td>
<td>-</td>
<td>correlation between the overstory and the environmental variables.</td>
</tr>
<tr>
<td>Student T-test</td>
<td>plots with distinct dominant oak species</td>
<td>best predictors</td>
<td>-</td>
<td>significant environmental differences between plots with distinct dominant oak species</td>
</tr>
</tbody>
</table>

First, the main gradients of woody species composition and abundance, and their relationship with edaphic factors, were examined by stepwise canonical correspondence analysis (CCA, ter Braak 1986). The stepwise option allowed the exclusion of highly redundant independent variables in the model, which could have caused model instability and/or over-estimation of the variance (Økland and Eilertsen 1994; Vandvik and Birks...
Second, the best spatial predictors were determined by another CCA of the species matrix constrained by the extended matrix of spatial data. Those best spatial predictors were then used as covariables in a partial CCA (pCCA; Økland and Eilertsen 1994), to determine the floristic variance explained by edaphic variables but non-spatially structured (Borcard et al. 1992). Third, the spatial component of floristic variance not shared with edaphic variables, was obtained by another partial CCA, in which the spatial variables were there predictors and the edaphic predictors (previously determined) were used as covariables (Borcard et al. 1992).

The basal area was calculated from the stem density and dbh, for each individual more than 1.30m high, and was considered a surrogate of the species overstory biomass. Any species that contributed more than 5% (on average) to the total basal area of the studied forest, and that had not the crown always covered by other individuals, was considered an overstory (or dominant) species. There were five species (A. unedo, E. arborea, Q. coccifera, Q. faginea, and Q. suber) under those conditions, which represented together near 97% of the total Basal area of the studied forest community. Spearman rank-correlation coefficients were calculated between the basal area of the five overstory species and: a) topographic variables, b) edaphic variables and c) community structure variables, to analyse the correlation between the overstory and the environmental variables.

Finally, the total basal area and the mean values of the environmental variables significantly related to the main floristic gradients, were compared among sites having different dominant oak species in the overstory. More precisely, that comparison was made between three plots where Q. faginea was absent while Q. suber had a significant contribution (an average of over 30 % of the total basal area) to the overstory biomass, and three plots where Q. suber was absent and Q. faginea had the highest proportion (average of 60 % of the total basal area) in the overstorey; additionally, the compared plots were chosen for having a negligible proportion (0.5% of the total basal area, on average) of the remaining oak species (Q. coccifera), and no significant differences between the relative contributions of the other overstory species (significance of t-tests were: \( p = 0.34 \) for A. unedo, and \( p = 0.90 \) for E. arborea). Mean comparisons were performed by Student t-test, after checking the data for normality (Shapiro-Wilks test).
and homocedasticity (Brown-Forsythe test), and applying transformations when necessary.

### 2.3.2. Recruits spatial patterns

To investigate the recruitment spatial pattern at the community level, the overall dispersion of all individuals irrespective of the species was calculated, using the modified Ripley’s K-function, \( L(r) = r - \sqrt{K(r)/\pi} \). This \( L(r) \) function makes interpretation easier (Diggle 2003), because when plotted against distance \( r \), negative values indicate clumping, and positive values indicate overdispersion (Fortin and Dale 2005).

The spatial pattern of each species (conspecific associations) was analysed, using the J-function (van Lieshout 2004, Baddeley et al. 2005). This function is identically equal to 1 for a completely random (uniform Poisson) point process, while deviations \( J(r) < 1 \) or \( J(r) > 1 \) indicate spatial clustering or spatial regularity, respectively.

Finally, the pattern of each recruit species relative to the remaining species (interspecific multivariate analysis) was calculated, using the ``type i to any type" multitype J function (Baddeley and Turner 2005).

J-function has many advantages over isolated F and G functions (Baddeley et al 1999, Thönnes and Lieshout 1999). It combines the nearest neighbor distance distribution function \( G(r) \), and the empty space function \( F(r) \), by simply taking the ratio

\[
J(r) = \frac{(1-G(r))}{(1-F(r))}
\]

, defined in Baddeley et al (2005). For interspecific multivariate analysis, the ``type i to any type" J function is described as

\[
J_{i}(r) = \frac{(1 - G_{i}(r))}{(1-F_{i}(r))}
\]

, where \( G_{i}(r) \) is the distribution function of the distance from a type i point to the nearest other point of the pattern, and \( F_{i}(r) \) is the distribution function of the distance from a fixed point in space to the nearest point of the pattern (Baddeley et al 2005).

All the statistics used go beyond simple testing for overall pattern, providing a detailed description of spatial dynamics (Moeur 1993, Thönnes and Lieshout 1999, Baddeley et al. 2000). The spatial patterns were investigated separately in each 32 x 10m plot, to guarantee stationarity.
By using Monte Carlo simulations, estimates of $K^*(r)$ or $J^*(r)$ were computed and 99% confidence envelopes were created, for comparison with the estimated empirical function. The maximum and minimum values of 100 random simulations were used to define the confidence envelope.

It is extremely important to clearly identify the null hypothesis, to avoid erroneous interpretations of the spatial structure and wrong biological conclusions (Diggle 1983, Goreaud and Pélissier 2003). The hypothesis of Complete Spatial Randomness (CSR) was the null hypothesis tested against the statistics of univariate patterns. The hypothesis of Independence between species, was the null hypothesis tested against statistics for multivariate patterns, i.e. statistics were tested conditionally on the spatial pattern of each species, keeping the location of one species unchanged, and shifting the points of the other species by a random vector, considering the study area as a torus (Goreaud and Pélissier 2003, Baddeley and Turner 2005).

2.3.3. **Regeneration niches**

Successfully established individuals of one tree species have passed through a chain of regeneration processes, including dispersal patterns, germination and emergence responses, and seedling growth and survival. However, the regeneration requirements (regeneration niche) of each tree species can be inferred from the analysis of the variables associated to the realized regeneration (i.e. the successfully established individuals).

The independency between the species of recruits in the understorey and the species of adult trees in the overstorey, was tested using the chi-square test (40 degrees of freedom for both plots considered together).

Stepwise canonical correspondence analysis (CCA, ter Braak 1986) was performed to study the relationships between the environmental variables and the recruitment of the studied species. According to the methods used for field collection of regeneration data - where each sample is an individual recruit - the CCA response matrix made of dummy variables representing each recruit’s species (category), but all the same theory of CCA applies to that nominal data (see ter Braak and Smilauer 2002, p. 61).

2.3.4. **Counteracting gradients**
Using the data collected in this thesis and data from a mixed-oak forest at the South of Spain (at *Los Alcornocales* Natural Park) the relationships between canopy density, litter accumulation and topsoil N and P availability in the understorey, were analyzed. Those relationships were firstly determined by correlation analyses. Afterwards, the path analysis by Shipley’s d-sep method (Shipley 2000, 2003; see Box 2 for details) was used, for a detailed inspection of the hypothesis that the overstorey canopy indirectly affects the topsoil N and P availability, throughout litter accumulation (i.e. higher canopy density causes higher litter accumulation, and this litter accumulation is responsible for the increase of N and P availability in soil).

Both separate and global causal models were tested, and separately for each forest site. The separate causal models considered the effect of litter accumulation over each nutrient (N or P) separately; the global models assumed that litter accumulation is the cause of the conjoint changes in N and P availability. For the global models, two different alternatives were considered, as shown in Figure 2.8: a) litter accumulation is the immediate cause of N and P changes, and b) litter influence on N and P availabilities is mediated by soil processes.

![Figure 2.8 – Schematic representation of two global models considered to test the hypothesis that the overstorey canopy indirectly affects the topsoil N and P availability, throughout litter accumulation. a: litter accumulation is the immediate cause of N and P changes; b: litter influence on N and P availabilities is mediated by the latent variable, soil processes.](image)

The magnitude of the increment of the average values of litter and soil nutrients at the extreme quartiles of the canopy density gradients (i.e. contrasting light environments), were also analyzed.
**Box 2**

Path analysis (also known as causal modeling) is the statistical technique that hypothesizes causal relationships among variables and tests the causal models with a linear equation system. The enormous advantage of the method is the possibility to infer causation with observational (non-experimental) data. In fact, it is an extension of the regression model, where regression is done for each variable in the model that is dependent on others that the model indicates as causes. The regression weights predicted by the model are compared with the observed correlation matrix for the variables, and a goodness-of-fit statistic is calculated, so that the best model can be determined.

The models are usually depicted in a figure in which single arrows indicate causation, and can involve either manifest variables, latent variables, or both. Manifest variables are those directly observable or measurable, and are usually represented in path analysis diagrams by enclosing the variable name within a square or a rectangle. Latent variables are those that cannot be observed directly and must be inferred from manifest variables - i.e. those implied by the covariances among two or more measured variables – and are usually represented by the variable name enclosed in an oval or circle.

Shippley (2000) made his own development of ‘d-sep’ (short term for ‘directed-separation’) test method - as an alternative to traditional maximum likelihood methods - that formally defines the logical relationships that can exist among variables in a directed graph (i.e. path models). The recent emergence of graphical modelling as a distinct statistical methodology has been extensively discussed, as shown by Whittaker (1990).

Although the d-sep method is not designed for testing models including latent variables (Shipley 2000), in our study a special case of the d-sep test was still applicable, assuming that N and P have correlated (not independent) errors (Shipley 2003).

### 2.3.5. Litter decomposition experiment

Non-linear mixed effects models were fit to the litter weight data, to determine the litter decomposition rate in each plot. Data was adjusted to an exponential curve, and particular attention was given to the fixed and random effects. A detailed inspection of the random errors was also performed, to evaluate the need to modulate their correlation or heterocedasticity.
2.4. Software, significance levels and other options

All over the thesis, computations and graphical display were made using the softwares
STATISTICA version 6 (StatSoft Inc. 2001), CANOCO for Windows version 4.5 (ter
Braak and Smilauer 2002), and MS Office Excel 2003. The R-language (R Development
Core Team 2006), along with the spatstat package (Baddeley and Turner 2005) and the
nlme package (Pinheiro et al. 2006), were also used in Chapters 3.2 and IV, respectively.
The stepwise option of CCA allowed the exclusion of highly redundant independent
variables in the model, which usually cause model instability and/or over-estimation of
the variance (Økland and Eilertsen 1994, Vandvik and Birks 2002). Manual selection and
Monte-Carlo permutation tests (with 9999 unrestricted permutations) under full model
were used.

Significance was fixed at the 0.05 level throughout all the studies of this thesis.
However, when repeated tests were carried out, two different approaches were used
(García 2004): i) in Chapter 3.1, only contributions having \( p < 0.01 \) were considered as
significant, while contributions having \( p < 0.05 \) (but \( p \geq 0.01 \)) were labeled as marginally
significant (ms); ii) in Chapters 3.2 and 3.3, type I error inflation was controlled by
applying a sequential Bonferroni procedure.

For analysing the floristic gradients (Chapter 3.1), log transformation (\( Y' = \log(Y+1) \))
and downweighting of rare species were applied to the species matrix. Besides, three
species (\( Cistus salvifolius \), \( Rhamnus alaternus \), and \( Juniperus \) sp.) were omitted because
each one occurred only once in one (not the same) plot.

For the K-function, an edge correction was applied using the “isotropic” method
(Baddeley and Turner 2005), while for the J-function the uncorrected estimates were used
and directly interpreted without edge correction (Thönnes and van Lieshout 1999,
Baddeley et al. 2000).

2.5. References

without edge correction. Statistica Neerlandica 54: 315-328.


III. Published papers referring to the observational study
3.1. The combined role of topography and overstory tree composition in promoting edaphic and floristic variation in a Mediterranean forest

S. Maltez-Mouro, LV. García, T. Marañón and H. Freitas

The combined role of topography and overstorey tree composition in promoting edaphic and floristic variation in a Mediterranean forest

Abstract This study aimed to address which factors, other than topography, contribute to the floristic variation of forested slopes. The natural forest studied is located in the Sudoese Alenejo e Costa Vicentina Nature Park (southern Portugal). We sampled topographic, edaphic, floristic and community structure variables along three bottom-top hillside transects. Multivariate analyses of soil variables (by PCA), and of woody species composition and floristic-environmental relationships (by CCA and rCCA) were performed. Environmental-floristic trends strongly associated with the elevation gradient were identified. At lower altitudes, the lowest species richness, the highest soil fertility, and the tallest and most dense (least available light) canopy occurred. The spatial variation in woody species composition and abundance was closely associated with Zn availability in the soil and litter groundcover, but these variables had significant spatial structure in the studied forest. The non-spatially structured species variance was better predicted by soil NO₃ and NH₄. The spatial variation of species data not shared with environmental variables was also calculated. We suggest that the influence of topographic gradient on the variation of edaphic variables and on the distribution and abundance of woody species was mediated by overstorey tree composition. Locally dominant tree species, in particular Quercus faginea and Quercus suber, may function as "ecosystem engineers" promoting environmental changes (i.e., Zn availability in soil, litter accumulation and light availability) that influence overall floristic variation.

Keywords Fertility gradient · Ecosystem engineer · Quercus faginea · Quercus suber · Soil Zn

Introduction A spatially heterogeneous distribution of forest species is produced by the interplay of the geophysical environment, physical processes, the impact of disturbances and the activities of organisms (Wiens 2000). It is well known that floristic structure and composition are influenced by a combination of mechanisms, such as density-dependent regulation of populations, competitive exclusion, regeneration dynamics, fire and other disturbances, and formation of canopy gaps (e.g., Honnay et al. 2001; Takahashi et al. 2001). The interactions between forest trees and their environment are bidirectional. On one hand, the spatial and temporal patterns of soil resources affect the local distribution and abundance of tree species and the performance of individual organisms (from seedlings to adult trees). These effects have important consequences for both community structure and ecosystem-level processes (Tilman 1988; Schlesinger et al. 1990). On the other hand, forest dynamics may be the outcome of overstorey-understorey interactions. The overstorey trees modify the environment underneath and influence species composition and plant growth in the understorey, while the differential success among plants and species in the understorey, in turn, affects the future overstorey composition (Flore et al. 1997).

On forested slopes, topography is a main ecological factor determining species spatial variability. Changes in the physical environment (from top to bottom of the slope) are associated with a complex environmental gradient, including changes in microclimate, water drainage and accumulation, and transport and accumulation of soil minerals. All these factors directly or indirectly affect the spatial patterns of plant species distribution and abundance (e.g., Hansa et al. 2000).
3.2. Spatial pattern analyses of the recruitment of four woody species in a Mediterranean oak forest

S. Maltez-Mouro, L.V. García, T. Marañón and H. Freitas

Recruitment patterns in a Mediterranean oak forest: the importance of the spatial component

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IV. Litter decomposition experiment
IV. Litter decomposition: an experiment using litterbags

4.1. Introduction

Litter decomposition describes one of the most important processes in the biosphere and an essential process in the functioning of forest ecosystems – the decay of organic matter and subsequent nutrient recycling (i.e. release of many mineral elements) (Berg et al. 2003).

The process of litter decomposition is controlled by both biotic and abiotic factors, but the relative importance of litter quality and climatic conditions in regulating decomposition appears to vary across biomes (Murphy et al. 1998). Besides, decomposition patterns are not always predictable from single-species dynamics (Gartner and Cardon 2004) and thus the real mixture found in the ecosystem’s litter must be considered in decomposition experiments. The litterbag experiment is convenient to classify communities relative to one another and gives an easily measured ‘index’ of actual decomposition processes.

Litter quality and quantity vary according to the forest structure and composition, along with light, water and temperature. The complex interaction between these factors, promote different litter decomposition rates and heterogeneous nutrients accumulation in the soil. Therefore, the species regeneration, survival and growth (regeneration processes), and ultimately the species abundance and distribution in the forest, is conditioned by the litter quality and quantity (Herrera 1994; Rossi y Villagra, 2003; Zamora et al. 2004; Broncano et al. 2004; Puerta-Piñero et al. 2006).

In the current chapter, we aimed to determine the litter decomposition rate at contrasting slope positions and forest communities. The main objective was to estimate if differences or affinities in litter decomposition rates relate to the topography, tree species composition and community structure (e.g. canopy density). These relationships between the litter decomposition and the overall species distribution and regeneration processes are essential information to implement proper management or conservation actions in the studied natural forest.
4.2. Materials and methods

4.2.1. Experimental design

A mesh was used to make ‘pockets’ which were filled with litter - litterbags. There is no clear answer in the literature to the best mesh size; it has to be a compromise between allowing in all fauna possible (c. 4-5 mm mesh) and errors due to loosing litter material through the holes and exchange of soil particles and other material. A mesh with 1.1 mm holes was used, which is a common mesh size in decomposition experiments (e.g. Hector et al. 2000).

Three litterbag harvests were made at six months intervals, i.e. the first harvest six months after placing litterbags in the field, the other after one year, and the last harvest after one year and a half. Eight litterbags per plot and per harvest were put in the field, thus a total of 48 litterbags were used.

The experiment started with the aboveground collection of recent litter, in particular all litter produced over an area or several areas in each plot, otherwise the content of different species and plant parts would not be representative of the community production. The collected material was sorted to assure that litterbags would include: litter produced during the current season; dead stems and woody plant parts; a representative mixture of the different plant parts and species (i.e. in proportion to the field production); green (not shredded by plants) and old litter. Seeds were not included and particular attention was taken in order not to crush or break the litter when collecting it. After that, the litter material was cut up into 5cm lengths, and material smaller than 5 cm was also cut once to warrantee the same cut ends in all litterbags (because more cut ends would lead to faster litter decomposition) (Quested and Garnier 2003).

The selected litter was dried at 20ºC for 4 days, in trays, turning it every so often, and not at high temperatures that would substantially alter the chemistry of the litter. Portions with 2g (+/- 0.1g) were put inside each litterbag, after very gently sieving the litter through a piece of the litterbag mesh, to avoid including small bits that would fall out from litterbags and give wrong records of mass loss.

Litterbags were placed back in the field and fixed on the surface (Figure 4.1), because it is the most realistic position for fresh litter, and the most common option for litterbag
placement (Hector et al. 2000). When placing bags, disturbance of plants and soil was minimized, and good contact between the soil/litter layer and the litter in the bag (without crushing the litter) was ensured. The bags were randomized with respect to the harvests and position, i.e. each bag had an equal chance of being in any harvest and any position in the plot.

![Figure 4.1 – Litterbag placed back in the field (surface placement).](image)

After retrieving the bags from the field, they were stored in the fridge for a maximum of 12 hours, and then each bag was carefully emptied. Plant roots inside the bag were removed along with all animals seen and any visible soil particles (e.g. Dukes and Field 2000). The harvested material was oven dried 60 °C for two days, and weighed. Four other litter portions (i.e. two from each plot) were oven-dried and weighted without being placed back in the field, to accomplish the actual mass loss not due to litter decomposition but only to the oven-drying method.

Great care in handling and transporting the litterbags at all stages was taken, handling them as little as possible to avoid breaking up the litter or losing fragments of litter. Analyses to determine the mineral content of litter were not performed as they were considered out of the scope of this chapter.

**4.2.2. Analysis of data**
Simple graphics were produced (using MS Office Excel 2003) to compare the litter weight loss along the three harvests and between the 2 plots.

To determine the litter decomposition rate in each plot, non-linear mixed effects models were fitted to data. Mixed-effects models, are a powerful tool for the analysis of experiments where some response variable is observed on multiple occasions (Pinheiro and Bates 2000). This may occur for each experimental unit, possibly under different experimental conditions, and hence, for longitudinal data or repeated measures data. These models incorporate the variability between subjects, by means of the expression of the models parameters in terms of both fixed and random effects, and therefore they allow to model covariance structures that need to be considered. It is the most appropriated approach for the litter decomposition data, where there might be a need to consider both the intra- and inter-individual variations. The use of a non-linear model, which parameters have a natural physical interpretation, allows a more parsimonious description of the data than using the competitor linear one (Pinheiro and Bates 2000).

Details on the commands and functions used to perform that model adjustment, using the R-language (R Development Core Team 2006) and the nlme package (Pinheiro et al. 2006), are shown in Appendix 7.1.

4.3. Results

4.3.1. Litter weight loss

As shown in Figure 4.2, litter loses considerable weight at each six months interval, reaching near 50% of the initial weight after only 12 months on the ground. The same graphic suggested the occurrence of higher litter decay in Plot U, which is the one with higher species diversity (including many shrubs), more light availability, less fertility, higher herbaceous ground-cover, and less litter groundcover (see Chapter 3.1). However, this was not confirmed after a detailed inspection of the best model for the litter decomposition, as shown in the next sub-section.
4.3.2. Finding the best model

The first inspection of the data, suggested the adjustment to an exponential curve, i.e.

$$\text{Weight} \sim A \times \exp(-B \times \text{Time})$$

where Weight is the litter weight measured at each Time (i.e. harvest), A is the intercept (i.e. initial weight), and B is the slope (i.e. decomposition rate).

Several models were considered and compared among each other, so that the very best model for litter weight loss could be determined (see details in Appendix 7.1). Both the intercept and the slope revealed to be significant fixed effects, while only the slope parameter had to be integrated in the model as a random effect. Furthermore, the intercept (initial weight) depended on (i.e. covariates with) the plot, but the slope (decomposition rate) did not.

A detailed inspection of the random errors showed no significant correlations between them. However, there was some heterocedasticity of the random errors, and therefore

Figure 4.2 – Litter weight loss shown as percentage of initial weight, for the three harvests at six months intervals. Average values calculated for the 8 litterbags collected in each harvest.
their covariance matrix was transformed, applying the exponential function to the variance (function “varExp” in Appendix 7.1).

The very best models reached are shown in Figure 4.3, and can be written as

\[
\text{Weight}_{\text{PlotD}} = 1.80 \times \exp(-0.050 \times \text{Time})
\]

\[
\text{Weight}_{\text{PlotU}} = 1.90 \times \exp(-0.050 \times \text{Time})
\]

From these models, we conclude that the decomposition rate was 0.05 month\(^{-1}\) in both plots, and the initial litter weight was 1.80 for plot D and 1.89 for plot U.

![Figure 4.3 – Exponential curves modelling the litter decomposition rate in each plot.](image)

### 4.4. Discussion

The use of non-linear mixed effects models to study the litter decomposition was appropriate because ecosystem processes occur non-linearly and irregularly (Schulze et al. 2002), and in particular litter mass varies exponentially with time.
On one hand, the slightly higher litter decay in the upper plot – with lower canopy density - is not in agreement with other studies, which have described a delay in litter decomposition due to the lower canopy density (e.g. Pardo et al. 1997). On the other hand, mass loss often increases when litter of different species are mixed (Gartner and Cardon 2004), and litter from the studied forest included more species in the upper plot than in the lower plot (Table 4.1).

Table 4.1 – Percentage of different plant parts and species found in the litter produced in each plot. The same proportions were used to fill each litterbag, so that they were representative of the community production.

<table>
<thead>
<tr>
<th>Plant Part</th>
<th>Plot U</th>
<th>Plot D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dead stems</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Herbs</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Arbutus unedo</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>Quercus coccifera</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Q. faginea</td>
<td>25</td>
<td>80</td>
</tr>
<tr>
<td>Q. suber</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>Smilax aspera</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Viburnum tinus</td>
<td>5</td>
<td>-</td>
</tr>
</tbody>
</table>

Although more tender leaves are expected to decompose faster (Pérez-Harguindeguy et al. 2000) and leaves rich in resistant components may have lower rates of decomposition (Moro and Domingo 2000), no significant differences were found in the decomposition rate down-slope vs. up-slope. In fact, a detailed inspection of the data showed similar litter decomposition rates in both contrasting slope positions. This could be expected because the effect of litter quality on mass loss is not always significant, due to the interactions between litter quality and site conditions (Garcia-Pausas et al. 2004).

The mineral content of litter was not analysed. However, according to data from other studies, if the initial litter nitrogen content, leaf toughness, toughness:nitrogen, and cutin:nitrogen were significantly different between plots, different decomposition rates should have been observed (Gallardo and Merino 1993, Murphy et al. 1998).

The different initial weights entered in each model, are due to the actual mass loss after oven-drying the samples and not to the litter decomposition, i.e. they are related to the water content of the litter material from each plot. These different water contents were expected, considering the distinct litter material produced in each plot (Table 4.1): litter in the lower slope is mainly composed by leaves of Q. faginea, which are soft and...
broadleaf, while in the upper-slope it includes leaves from sclerophyllous plants and shrubs, which are more rich in hard components.

The litter decomposition rate determined in this study (-0.05 month⁻¹, i.e. -0.6 year⁻¹) can be compared with the decomposition rates referred in other studies; for example, -0.85 year⁻¹ in a natural tropical forest at Pau-Brasil (Gama-Rodrigues 2003), -0.55 year⁻¹ in an ecosystem dominated by shrub legumes, -0.34 year⁻¹ in a grassland (Oliver et al. 2002), and -0.26 year⁻¹ down to -0.17 year⁻¹ in mediterranean-type forests at southern Tuscany, from deciduous through sclerophyllous to coniferous vegetation (Wesemael and Veer 1992). Figure 4.4 illustrates the increment of litter decomposition rate from one ecosystem to another, showing that the studied Mediterranean natural forest has the second higher decomposition rate, just after a Tropical forest.

![Figure 4.4](image)

Figure 4.4 – Schematic representation comparing the litter decomposition rate determined in different ecosystem types. The studied forest (in italics) has the second higher rate of decomposition when compared to data from Gama-Rodrigues (2003), Oliver et al. (2002), and Wesemael and Veer (1992).

The forest canopy influences several microclimatic factors, thus it has a large influence on nutrient cycling. Besides, canopy characteristics affect the amount and composition of leaf litter produced, which largely determines the amount of nutrients to be recycled and the resulting nutrient availability (Prescott 2002). However, the effects of tree species on soil nutrient availability can be better predicted from the mass and nutrient content of litter produced than from the litter decay rate (Prescott 2002), which is limited by carbon substrates rather than by nutrient content (Murphy et al. 1998). Therefore, more
comparative studies are necessary to find a decomposition model suitable to different floras (Vaieretti et al 2005).

The faunal activity could also be considered to influence the litter decomposition, but temperature and moisture conditions, and the chemical and physical nature of the litter, are frequently more important controlling litter decomposition than the distribution of faunal activity (Prescott 2002, Garcia-Pausas et al. 2004).

Further analysis should be made to determine the exact litter contents, and to investigate the relationships between litter components, decomposition rates, soil nutrients availability, community structure, and species distribution in the studied forest.

4.5. References


V. Published paper comparing the field data of this thesis with data from a mixed-oak forest at the South of Spain
5.1. Counteracting gradients of light and soil nutrients in the understorey of Mediterranean oak forests

L.V. García, S. Maltez-Mouro, Ignacio M. Pérez-Ramos, H. Freitas, and T. Marañón

Counteracting gradients of light and soil nutrients in the understorey of Mediterranean oak forests

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VI. Discussion
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6.1. General discussion

Natural forests are rare and disperse at the south of Portugal, where they persist mainly in steep slopes. This is the case of our studied site, and therefore the first objective of this thesis was to improve the knowledge on the effects of the topographic gradient on the ecology of forest communities. Results (Chapter 3.1) showed that the edaphic properties change along the main slope gradient, with a top-bottom increase of soil fertility. That was paralleled by a floristic gradient, from an upper-slope community dominated by arborescent shrubs and with isolated trees of *Quercus suber*, towards a bottom-slope community dominated by *Quercus faginea* and with few shade-tolerant shrub species. These edaphic and floristic gradients corresponded to an overall structural heterogeneity that also changes along the slope, with the maximum tree height, canopy density and litter accumulation increasing from top to bottom-slope, and the species richness increasing up-slope. The referred gradients have been shown in many forest types (e.g. Hanba et al. 2000, Hirayama and Sakimoto 2003, Son et al. 2004, Benayas et al. 2005, Ajbilou et al. 2006), as they have been described for other Mediterranean forests (Teo: which Reference do you advise to use here?!), but a detailed description was missing for the persisting rare Mediterranean forests at the south of Portugal.

The floristic heterogeneities correspond to structural heterogeneities at the community level and specific variations in the overstory species composition. We investigated if, in turn, the overstory heterogeneity itself have some effects on the floristic composition or the overall forest ecosystem. Results revealed that independently from the previously described top-bottom gradients, a close relationship was evident between the dominant oak species and the edaphic variables. We concluded that dominant oak species may act as ecosystem engineers, i.e. they modulate the light and edaphic resources from which the other plant species are dependent (Jones et al. 1997).

In summary, topography has indirect influence on the floristic composition through its influence on edaphic variables. Besides, the functional heterogeneity related to the overstory species composition also influences the (edaphic) environmental variables, thus the whole floristic composition and community structure (Figure 6.1).
Regarding the regeneration processes, results (Chapter 3.2) showed the existence of inter-species differences, which is in agreement to what has been found in other Mediterranean forests (e.g. Pérez-Ramos and Marañón 2005). Besides, some species of recruits were not randomly distributed, as it is the case in most terrestrial ecosystems (Perry et al. 2002). These two conclusions were explained by the interplay between microclimatic conditions and physiological trade-offs (i.e. micro-environmental filters and environmental requirements of each species) and by the dispersion strategies of each species; in particular, the dependence on the mother trees was the most determinant factor to explain the recruits spatial patterns, as it has been shown in other studies (Herrera et al 1994, Jordano and Herrera 1995).

Light, litter, soil moisture, and the canopy species themselves, were important to explain the recruits’ distributions and associations. Not only all these factors depend on the functional heterogeneity of the ecosystem, but also results suggested that the recruitment patterns were influenced by the overstory structure and composition. Therefore, a new hypothesis was drawn, stating that the overstory canopy have direct and indirect effects on recruits (Figure 6.2), mediated by processes of facilitation and inhibition (Holmgren et al. 1997).
The spatial structure of abiotic and biotic factors, populations, communities, or biodiversity has often been treated either as a problem or ignored. However, an increasing awareness of the importance of space in ecology is apparent (Wagner 2003). In fact, the spatial structure might be caused by environmental factors or community-level processes, thus it is essential to take it into account, to gather information on the biological processes behind any ecological community (Maestre et al. 2003). Results showed that, beyond the direct effects of the environment and the direct and indirect effects of the overstory components, the spatial component was also of major importance in the recruitment process. In fact, 14% of the total species variance and a 55-60% of the explained recruits variance, were spatially structured and independent from the environmental or canopy variables.

An unexplained proportion of floristic variance and recruitment patterns persisted in each approach, even if the micro-environmental and overstory components explained most of those heterogeneities. Although we must hypothesize that some relevant factors may have been overlooked, it has been recognized that large amounts of stochastic variation are always present in any community structure and dynamics (Borcard et al. 1992).

The next step of the thesis was to study the process of litter decomposition, which describes an essential process in the functioning of the forest ecosystems (Berg et al. 2005). Results showed that the water content of litter was different between the upper-slope and bottom-slope communities. This was expected because distinct litter material
was produced along the slope, with the upper-slope material including leaves from sclerophyllous plants and shrubs (richer in hard components), and the lower slope material being mainly composed by leaves of *Q. faginea* (soft and broadleaf).

The litter decomposition rate showed to be the same in both the upper-slope and bottom-slope communities of the studied forest. The reached value was -0.05month⁻¹, which is equivalent to -0.6year⁻¹. Comparing this value with the ones obtained in other studies (Gama-Rodrigues 2003, Oliver et al. 2002, and Wesemael and Veer 1992), we conclude that the studied natural forest has a considerable high decomposition rate, only lower than the rate determined for a tropical forest.

It has been demonstrated that the process of litter decomposition is controlled by both biotic and abiotic factors, and by litter quality and quantity (i.e. chemical composition) (e.g. Gallardo and Merino 1993, Murphy et al. 1998, Moro and Domingo 2000, Pérez-Harguindeguy et al. 2000, García-Pausas et al. 2004). Therefore, if the forest canopy influences several microclimatic factors and affect the amount and composition of leaf litter produced, it should influence the litter decomposition rate and the resulting nutrient availability (Prescott 2002). Finally, the forest canopy will thus condition the regeneration processes, and ultimately the species abundance and distribution in the forest (Herrera 1994, Rossi y Villagra 2003, Zamora et al. 2004, Broncano et al. 2004, Puerta-Piñero et al. 2006).

The effects of the forest canopy on the growth and survival of tree seedlings in the understory, have long been recognized, and can be divided in two categories: direct effects, through changes on the availability of light and water (Sack *et al.* 2003, Quero *et al.* 2006, Sánchez-Gómez *et al.* 2006), and indirect effects, which are mediated by litter and ultimately by soil nutrients (Rossi y Villagra 2003, Broncano *et al.* 2004, Puerta-Piñero *et al.* 2006). *Chapter 5.1* focused on the importance of considering both the indirect and direct effects of the overstory over the understory and, in particular, the need to distinguish between them to assure that the less conspicuous ones are not obscured by the others.

Results (*Chapter 5.1*) showed that the canopy density, litter accumulation and nutrient availability, covariate. However, the overstory canopy had counteracting effects over light and nutrients. These counteracting gradients condition the regeneration processes
and species distribution in the forest, depending on their ability to cope with limiting light versus soil nutrients availability.

6.2. Conclusions
The knowledge achieved in this thesis, concerning the forest heterogeneities and gradients, the recruitment patterns and inter-species differences of regeneration, the rates of litter decomposition, and the complex covariation of different resources (e.g. light and soil nutrients), is important to optimize conservation actions of natural Mediterranean forests that persist nowadays.

On one hand, this study showed that some management actions should be applied to the remaining natural mixed-oak forests, and particularly regarding the evident ‘natural’ replacement of remnant old and injured cork oaks by vigorous and young semi-deciduous oaks. On the other hand, those management actions must take into account the ‘natural’ heterogeneities of the forest community, such as: the higher abundance and recruitment success the semi-deciduous oak (*Q. faginea*) in the lower-slope, along with the higher abundance and relatively good recruitment of the evergreen cork oak (*Q. suber*) in the upper-slope; the soil fertility gradient; the species richness gradient; the light availability gradient; the overall complex covariation between different gradients; and the inter-species different regeneration patterns.

Finally, this thesis showed that special attention should always be paid to the overstory structure and composition, as it directly and indirectly changes the micro-environment and resources (e.g. temperature, moisture, light quantity and quality, and litter quantity and quality), and therefore plays a major role on the recruitment process and overall community structure and dynamics.

6.3. Suggestions for further work

It is often the case with scientific investigations that more questions than answers are produced.

Does your work suggest any interesting further avenues?
Are there ways in which your work could be improved by future workers?
What are the practical implications of your work?
6.4. References


en un mundo cambiante. Ministerio de Medio Ambiente, Valladares, F. 2004 (ed.).
EGRAF, S.A., Madrid.
VII. Appendices
VII. Appendices

7.1. Commands and functions used in R Console to modulate the litter decomposition rate

library (nlme)
library (lattice)
trellis.device()
background<-trellis.par.get("background")
background$col<="transparent"
background<-trellis.par.set("background",background)

DataL<-read.table("/Users/Sara/PhD/Analises-LitterBags/Dados.txt", head=T)
names (DataL)
attach (DataL)

TIME <- DataL$TIME
PLOT <- factor(DataL$PLOT)
BAG <- DataL$BAG
WEIGHT <- DataL$WEIGHT

Data <- data.frame (TIME, PLOT, BAG, WEIGHT)

DataG<-groupedData(WEIGHT~TIME | BAG, outer=~PLOT, Data,
labels=list(x="Harvest", y="Weight"),
units=list(x="(months)", y="(g)"))

plot (DataG,outer=~PLOT,layout=c(2,1),aspect=1.5)
plot (DataG,outer=~1)

# Perception of the curve shape
plot (TIME, WEIGHT)
lines (lowess (TIME, WEIGHT, 1), col="blue")
# It is an exponential growth

# Separate nonlinear fits by groups
Glist <- nlsList(WEIGHT~A*exp(-B*TIME),DataG,start=c(A=1.832,B=0.00459))
Glist
summary(Glist)

plot (intervals(Glist))
pairs(Glist,id=0.1)
plot(Glist)
plot(Glist,BAG~resid(.),abline=0)

# Model adjustment
options (contrasts=c("contr.treatment",ordered="contr.poly"))
contrasts (DataG$PLOT)

M1 <- nlme(WEIGHT~A*exp(-B*TIME),data=DataG,fixed=A+B~1,random=A+B~1,start=list(fixed=c(A=1.832,B=0.00459)))

summary (M1)
anova (M1)

# Reducing the number of random effects
M4 <- update (M1, random=B~1)
anova (M1, M4)

M5 <- update (M1, random=A~1)
anova (M1, M5)

anova (M4,M5)

# M4 is the best
intervals(M4)
summary(M4)

# Quality of the model adjustment
plot (M4, id=0.05)
plot (M4, resid(.)~fitted(.)|PLOT, id=0.05)
plot (M4, BAG~resid(.), abline=0)
plot (M4, resid(.)~TIME, id=0.05, abline=0)

# Check of Normality
qqnorm (M4, ~resid(.), type="p")

rM3 <- resid (M4)
rM31 <- as.vector (rM3)
shapiro.test (rM31)

# Observed versus Adjusted values
plot (M4, WEIGHT~fitted(.), id=0.05, abline=c(0,1))
plot (M4, WEIGHT~fitted(.)|PLOT, id=0.05, abline=c(0,1))

# Check of correlation
ACF (M4)
plot (ACF (M4), alpha=0.05)
acf (resid (M4))

# Modeling the Heterocedasticity
M6<-update(M4,weights=varConstPower())
anova(M4,M6)
M61<-update(M4,weights=varPower())
anova(M4,M61)
M62<-update(M4,weights=varExp())
anova(M4,M62)
M63<-update(M4,weights=varExp(form=~TIME))
anova(M4,M63)

# M62 is the best
plot (M62, resid(.)~TIME, id=0.05, abline=0)
plot (ACF (M62, resType="n"), alpha=0.05)
qqnorm(M62)
plot(comparePred(M4,M62))

# Addition of covariables
M5<-update(M62,fixed=list(A~1,B~PLOT),start=list(fixed=c(1.83,0.04,0.04)))
summary(M5)
anova(M62,M5)
anova(M4,M5)
M5new<-update(M62,fixed=list(A~PLOT,B~PLOT),start=list(fixed=c(1.83,1.83,0.04,0.04)))
summary(M5new)
anova(M5new)
anova(M62,M5new)
M5new1<-update(M5new,fixed=list(A~PLOT,B~1),start=list(fixed=c(1.83,1.83,0.04)))
summary(M5new1)
anova(M5new1)
anova(M5new1,M5new)

# M5new1 is the best

# Final Model: M5new1
plot(TIME, WEIGHT, xlab="Harvest(months)", ylab="Weight (g)", type="n")
points(TIME[PLOT=="D"], WEIGHT[PLOT=="D"], pch=1)
points(TIME[PLOT=="U"], WEIGHT[PLOT=="U"], pch=16)
time<-seq(0,18,0.01)
yD<-1.800*exp(-0.0503536*time)
yU<-1.890*exp(-0.0503536*time)
lines(time,yD)
lines(time,yU,lty=2)
legend(14,1.8,c("Plot D","Plot U"), pch=c(1,16), lty=c(1,2), bty="n")