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EFFECTS OF LAND USE CHANGE ON PLANT COMPOSITION AND ECOSYSTEM FUNCTIONING IN AN EXTENSIVE AGRO-PASTORAL SYSTEM: PLANT FUNCTIONAL TRAITS AND ECOSYSTEMS PROCESSES

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ABSTRACT

As a consequence of the industrial and socio-demographic changes of the last decades, the characteristic land use practices of extensive agro-pastoral systems in the region of Alentejo, Southern Portugal are being gradually abandoned and the consequences are still little understood. Land use changes are important drivers of environmental degradation, modification and fragmentation of habitats with the subsequent alterations of global carbon and hydrological cycles, global and regional climate, and decline in biodiversity.

This work aims to contribute to a better understanding of the effects of abandonment in ecosystems with a long history of human management, as is the case of the agro-pastoral systems of Southern Portugal. Three land use categories were selected to represent a decrease in land use intensity ('grazing', 'intermediate succession' and advanced succession'). Following abandonment, secondary succession is expected to occur with consequent changes in soil characteristics and vegetation composition and structure. Because plant species differ in their functional traits and in their effects on ecosystem processes, an effect of land use change at this level may also be expected. Plant traits relate to universal plant functions of growth (e.g. light and nutrient acquisition, water use efficiency) and persistence (e.g. recruitment, dispersal, defence against herbivores and other disturbances). They provide a widely applicable framework for interpreting community shifts along environmental gradients, including secondary succession.

The main objectives were to (1) identify changes in vegetation composition and structure in response to decreasing land use intensity; (2) Identify functional groups and changes in species traits in response to decreasing land use intensity; (3) Identify the effect of decreasing land use intensity in key ecosystem processes such as decomposition and above-ground net primary productivity (ANPP).

Overall, the results from the present study showed that abandonment resulted in changes in species composition and richness, in plant functional traits and functional groups and in ecosystem processes (biomass, ANPP and decomposition). Secondary succession resulting from abandonment of grazing showed stronger changes in vegetation composition and structure than in soil characteristics. Among the soil chemical properties tested, only phosphorus, carbon and organic matter were affected by land use change. Phosphorus decreased with abandonment while organic matter and carbon showed an increase. Species richness decreased sharply after land abandonment, and this was associated with a strong turnover in species composition from grazed to abandoned sites as the vegetation changed from annual herbaceous to shrub-dominated communities. One single species (*Cistus ladanifer*) accounted for more than 50% of relative cover in the areas abandoned for a longer time, suggesting that this species might have an important role in possible changes in ecosystems processes.

The species that colonized the different land use categories differed in plant functional traits. Therophyte life form, short canopy height, high specific leaf area (SLA), low leaf dry matter content (LDMC) and small seeds with dispersal structures were dominant at grazed plots. Within abandoned plots, chamaephytes dominanted at 'intermediate succession' plots and decrease in favour of nanophanerophytes in 'advanced succession' plots. Canopy height, LDMC and seed mass increase with abandonment time while SLA decreased. Functional response groups were found by combining life form and SLA and these were found to be sufficient to describe vegetation change. Therophytes with medium SLA were the dominant functional group in grazed areas, while nanophanerophytes with medium or low SLA were associated with later phases of abandonment. At intermediate stages of succession the dominant group was chamaephytes with medium SLA but functional diversity was highest as all the groups, except hemicriptophytes with medium SLA, were represented.

Our study showed an increase in total above-ground biomass with abandonment indicating a positive effect of the shift to woody vegetation on total above-ground biomass. ANPP significantly increased in plots abandoned for longer time. This increase was strongly related with the increase in the cover of *Cistus ladanifer*. This is a pioneer species that colonises degraded areas and forms one of the first stages of succession of woody communities. Under favourable conditions, *C. ladanifer* can grow fast and attain large amounts of biomass in a short time and quickly spreads over recently disturbed areas.

Regarding decomposition, shrub litter was found to be higher than herbaceous litter in nutrient content, especially nitrogen, which seemed to favour higher initial decomposition rates but lower decomposition rate in the longer term. Overall, decomposition was slower in abandoned than in grazed plots and this was positively correlated with the content of cellulose and hemicellulose of initial litter. Lower rates of decomposition were also found to be related to the increase in LDMC, a trait strongly linked to physical attributes of the leaves.

Resumo

Em consequência da rápida evolução da indústria e alterações sócio-demográficas das últimas décadas, assistiu-se a um abandono gradual das práticas de agricultura e pastorícia extensivas típicas dos sistemas agro-silvo-pastoris do Alentejo. Os efeitos desse abandono nas características da vegetação e no funcionamento do ecossistema são ainda pouco conhecidos. As alterações no uso do solo conduzem à perda, modificação e fragmentação de habitats e a consequentes alterações dos ciclos globais da água e do carbono, do clima regional e global, bem como a uma diminuição da biodiversidade.

Este trabalho pretende contribuir para um melhor conhecimento dos efeitos do abandono de práticas de gestão extensivas em áreas em que estas são praticadas há séculos, como é o caso dos sistemas agro-pastoris do sul de Portugal. Para tal, foram seleccionadas três áreas representativas de diferentes intensidades de uso do solo ('pastoreio', 'sucessão intermédia' e 'sucessão avançada'). O abandono das práticas agro-pastoris dá início a um processo de sucessão secundária, o qual, é geralmente, acompanhado por alterações físico-químicas do solo bem como por alterações da composição e estrutura da vegetação. As espécies vegetais diferem quer nos atributos funcionais que as caracterizam quer no modo como influenciam o funcionamento dos ecossistemas. Os atributos funcionais estão relacionados com aspectos como o crescimento (aquisição de luz e nutrientes, eficiência no uso da água) e a sobrevivência (dispersão, regeneração, protecção contra herbívoros e outras perturbações). Estes permitem interpretar variações nas comunidades vegetais ao longo de gradientes ambientais, nos quais se pode incluir a sucessão secundária. Em resposta ao abandono, foram objectivos deste trabalho identificar: (1) alterações na composição e estrutura da vegetação; (2) grupos funcionais e variações nos atributos funcionais; (3) efeitos na biomassa e produtividade primaria da parte aérea, bem como na taxa de decomposição da folhada.

Os resultados deste estudo demonstraram que o abandono tem como consequências a alteração da composição e número de espécies, dos atributos funcionais e grupos funcionais, bem como das propriedades do ecossistema estudadas (biomassa e produtividade primária da parte aérea, e decomposição da folhada). Os efeitos do abandono de práticas agro-pastoris extensivas nas áreas de estudo foram mais visíveis na composição e estrutura da vegetação do que nas características do solo. De entre os parâmetros do solo determinados, apenas se registaram alterações no conteúdo em fósforo, carbono e matéria orgânica. Observou-se uma diminuição de fósforo e um aumento de carbono e matéria orgânica em função do tempo de abandono. A riqueza específica diminuiu significativamente após o abandono e foi acompanhada por uma considerável alteração da composição florística, sendo que comunidades maioritariamente compostas por herbáceas anuais foram substituídas por comunidades arbustivas. Nas áreas de 'sucessão avançada' há dominância de uma espécie, *Cistus ladanifer*, responsável por mais de 50% da cobertura relativa, o que sugere que esta espécie pode ter um efeito importante no funcionamento destes ecossistemas.

As espécies presentes nas diferentes categorias de uso do solo apresentaram diferenças nos atributos funcionais. Atributos associados às áreas com pastoreio incluem, forma de vida terófita, plantas de pequeno porte, área específica da folha (SLA) elevada, teor de matéria seca da folha (LDMC) baixo, sementes pequenas com mecanismos de dispersão. Relativamente às áreas abandonadas, os caméfitos dominam nas áreas de 'sucessão intermédia', diminuindo em favor dos nanofanerófitos nas áreas de 'sucessão avançada'. Os atributos altura da copa, LDMC e massa da semente aumentaram com o tempo de abandono enquanto a SLA diminuiu. Através da combinação de dois atributos, forma de vida e SLA, foi possível identificar grupos funcionais de resposta. Terófitos com SLA média foram o grupo funcional dominante nas áreas de pastoreio, enquanto os nanofanerófitos com SLA média ou elevada foram o grupo dominante nas áreas de 'sucessão avançada'. Em fases intermédias da sucessão

o grupo dominante foram os caméfitos com SLA média, mas a diversidade de grupos funcionais foi mais elevada do que nas restantes categorias pois todos os grupos, com excepção de hemicriptófitos com SLA média, estavam representados.

O estudo apresentado mostrou um aumento da biomassa da parte aérea total com o aumento do tempo de abandono indicando um efeito positivo da substituição de espécies herbáceas por espécies arbustivas na biomassa aérea total. A produtividade primária líquida da parte aérea foi significativamente mais alta nas áreas abandonadas há mais tempo. Este aumento deveu-se em grande parte ao aumento da cobertura de *Cistus ladanifer*. Esta espécie é pioneira na colonização de solos degradados e forma um dos primeiros estados da sucessão de comunidades arbustivas. Em condições favoráveis esta espécie pode acumular grandes quantidades de biomassa em pouco tempo e rapidamente colonizar áreas recentemente perturbadas.

A análise da qualidade inicial da folhada mostrou valores mais elevados de azoto na folhada das áreas abandonadas, o que parece ter contribuído para a decomposição rápida destes tipos de folhada em fases iniciais e mais lenta em fases mais tardias. Em termos gerais, a decomposição da folhada foi mais lenta nas áreas abandonadas do que nas áreas com pastoreio. Os resultados mostraram uma correlação positiva entre a taxa de decomposição e o conteúdo inicial de celulose e hemicelulose da folhada. Adicionalmente, registou-se uma correlação negativa entre a taxa de decomposição e a LDMC, um atributo relacionado com a composição estrutural das folhas.

Contents

INTRODUCTION

Land use changes are important drivers of environmental and biodiversity changes worldwide (Vitousek and Mooney 1997, Sala *et al.* 2000), leading to the loss, modification and fragmentation of habitats with the subsequent alterations of global carbon and hydrological cycles, global and regional climate, and decline in biodiversity (Foley *et al.* 2005).

In Europe, land abandonment and a decrease of land use intensity is a widespread tendency that is expected to increase, particularly in lands that are economically marginal for production (Puidgefábregas and Mendizabal 1998, Rounsevell *et al.* 2005), such as parts of the regions with Mediterranean climate.

Mediterranean landscapes are the result of the interaction of a long history of anthropogenic disturbances (cultivation, grazing, timber and fuel wood) with natural disturbances (such as fire, floods and extreme droughts) and a variable climate (Lavorel and Richardson 1999, Gallego-Fernández *et al.* 2004). The agro-silvo-pastoral systems of South Portugal are an example of such complex interactions (Pinto-Correia and Mascarenhas 1999). They likely originated from mixed forests of holm and cork oak and other species with persistent leaves and are the result of centuries of human management (Capelo and Catry 2007). Their long management history implies that land use legacies are an essential component when attempting to understand their current dynamics and that they should be considered when interpreting community and ecosystem response to current land use changes (Quétier *et al.* 2007).

For a local biota, the regional species pool consists of a specific set of species that represent the outcome of history, biogeography and evolutionary processes (Naeem and Wright 2003). Ecosystems respond to shifts in land use by changes in structure (*e.g.* plant cover, plant functional type composition) and function (*e.g.* primary production, decomposition, nitrogen dynamics, Aguiar *et al.* 1996). Particularly in areas with long history of management, land abandonment may be expected to cause modifications in the number and

identity of the species present, community structure, and variations in stocks and fluxes of carbon and nitrogen (Chapin III *et al.* 2000). Understanding how changes in plant communities, such as species richness and composition, influence ecosystem processes requires an understanding of the functional traits of the species involved (Chapin III and Walker 1997, Chapin III *et al.* 2000), as well as an understanding of the effects of land use change on plant functional traits and on ecosystem processes (Fig. 1).
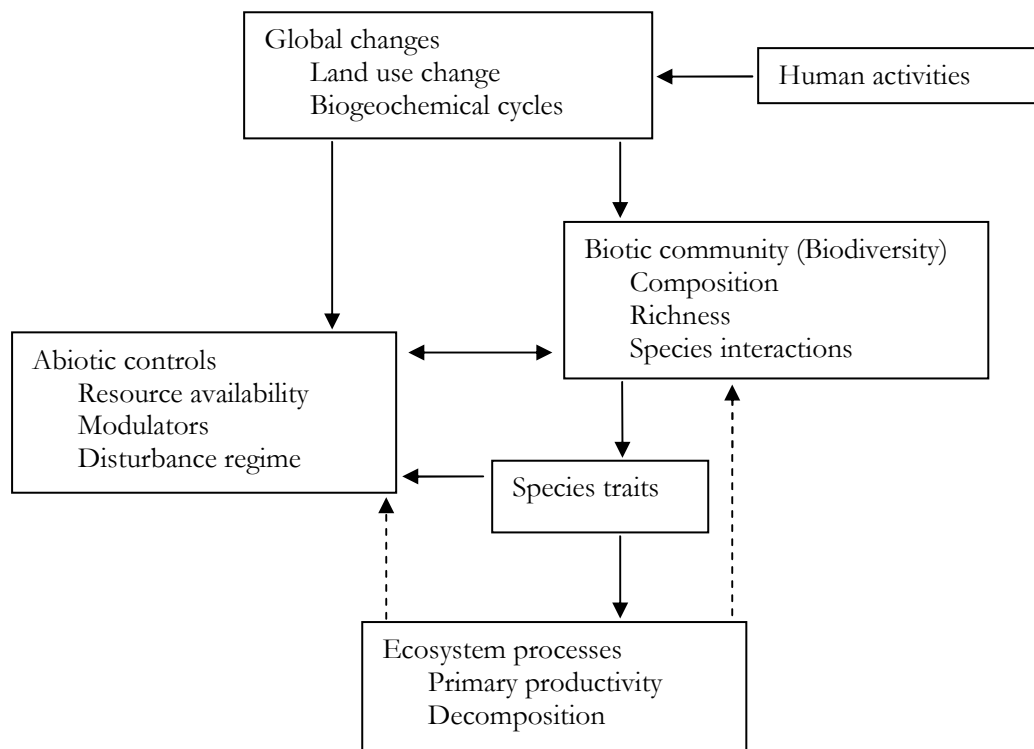
Figure 1 - Feedbacks between human activities, global change, and biotic and abiotic controls on ecosystem processes. Human activities cause environmental and ecological changes of global significance. These global changes contribute to changing both the biodiversity and the abiotic controls. Various aspects of the biotic community influence the range and proportion of species traits. The traits can indirectly affect ecosystem processes by altering the abiotic controls or directly affect ecosystem processes. Altered ecosystem processes can feedback to further change biodiversity either directly or through further changes in the abiotic controls (dotted lines). Modulators are abiotic conditions that influence process rates (*e.g.* soil temperature and moisture) but are not directly consumed in the process (Chapin III 2003). This figure is modified from (Chapin III *et al.* 2000 and Hooper *et al.* 2005).

PLANT FUNCTIONAL TRAITS AND PLANT FUNCTIONAL GROUPS

Plant functional traits are morphological, physiological or phenological features measurable at the individual level, from the cell to whole-organism level, without reference to the environment or any other level of organization (Woodward and Kelly 1997, Violle *et al.* 2007). A trait may be either continuous or categorical, it may show different attributes along environmental gradients or through time, and it can either be a response and/or effect trait (Diaz and Cabido 2001, Violle *et al.* 2007). It relates to universal plant functions of growth (*e.g.* light and nutrient acquisition, water use efficiency) and persistence (*e.g.* recruitment, dispersal, defence against herbivores and other disturbances, Weiher *et al.* 1999). Plant functional traits provide a widely applicable framework for interpreting community shifts along environmental gradients (Hodgson 1999 Cornelissen 2003, Lavorel 2007). The analysis of plant functional trait response to environmental variation, as well as of their effects on ecosystem function, has been guided by the recognition that plants like all living organisms are constrained for performing alternative functions simultaneously with a limited amount of available resources (Weiher *et al.* 1999, Westoby*, et al.* 2002). Therefore, plants need to establish trade-offs between different functions in order to adapt to the environment. An example is the trade-off between rapid acquisition of resources and the conservation of resources, often associated to specific leaf area (SLA, Westoby *et al.* 2002). Species with high SLA tend to also have higher leaf nitrogen content, higher photosynthetic capacity, and generally faster turnover of plant parts that permit flexible response to the spatial patchiness of light and soil resources giving short-term advantage over species with low SLA (Garnier *et al.* 1997, Reich *et al.* 1997, Westoby *et al.* 2002). A second example is the fecundity and seed mass trade-off, which relates to establishment opportunities and success in the face of hazards. Seed mass and fecundity are negatively correlated: plants with small seeds have higher fecundity, while species with large

seeds generally germinate better and have seedlings with better survivorship (Westoby 1998, Turnbull *et al.* 1999) but produce fewer seeds.

Plant functional groups are non-phylogenetic assemblages of species based on common sets of traits which can be affected in a similar way by environmental factors (*functional response groups*) and/ or have similar effects on major ecosystem processes (*functional effect groups,* Diaz and Cabido 2001). Plant functional groups have been seen as a tool for the simplification of floristic complexity in global vegetation models and for monitoring the effects of global change or management on vegetation distribution and ecosystem processes (any process measured at the ecosystem level, such as above-ground biomass, decomposition; Lavorel *et al.* 2007, Violle *et al.* 2007). Although this classification can be quite useful, in practice, defining the different functional groups is often difficult because this requires arbitrary decisions as to where a boundary between groups lies (Hooper *et al.* 2005). Additionally, traits and functional groups that are of interest for one ecosystem process or response to one environmental factor may not be of interest for others (Lavorel and Garnier 2002). Finally, traits that determine how a species responds to disturbance or environmental change (response traits) may differ from those that determine how that species affects ecosystem processes (effect traits, Diaz and Cabido 2001). Understanding the links between functional response traits/groups, and effect traits/groups remains a significant challenge (Hooper *et al.* 2005, Lavorel *et al.* 2007).

According to Lavorel *et al.* (2007), continuous traits should be used for the classification of plants into functional groups. The focus should be on functional traits that can together represent the key response and effects of vegetation at various scales from ecosystems to landscapes, biomes and continents, and that can be used to develop a satisfactory functional classification for global scale modelling of ecosystems. Therefore, from all the possible traits within a individual, those of interest for functional classification should fulfil four criteria: (1) have some relationship to plant function, (2) be relatively easy to observe and quick to

quantify, (3) be measured in a way that can be standardized across a wide range of species and growing conditions (4) have a consistent ranking – not necessarily absolute constant values – across species when environmental conditions vary (Hodgson *et al.* 1999, Weiher *et al.* 1999, Lavorel *et al.* 2007). A list of plant traits was proposed by Weiher *et al.* (1999) and the links between plant traits and their response to environmental filters and their effects on ecosystem processes were presented by Lavorel and Garnier (2002). Links between traits relevant for this study and their response to environmental filters and/or effect on ecosystem processes are summarized in Table 1.

SPECIES TRAITS AND ENVIRONMENTAL GRADIENTS

Environmental factors can be seen as filters that constrain which species from a regionally available pool can persist at a site. Filtering operates by changing the proportion of the different genotypes of a community and it strongly determines which traits and functions can survive at any particular site. Major environmental filters include climate, disturbance regime and biotic interactions (Díaz *et al.* 2007). The Mediterranean region has a long history of disturbances such as fire, grazing and ploughing, with an important effect for the Mediterranean vegetation.

Several traits have been related to disturbance (*e.g.* Noy-Meir *et al.* 1989, Fernández-Alés *et al.* 1993, Landsberg *et al.* 1999, Pausas and Bradstock 2007) but few general patterns have been found. Disturbed habitats seem to favour ruderal species, which are short-lived plants of rapid growth investing a large proportion of resources in reproduction and devoting little resources to defence (Grime 2001). Plant species tolerant to soil disturbance by ploughing are usually short and have prostrate habit or flat rosettes, high fecundity and a small dormant seed pool

Table 1 – Relevant traits and their links to environmental response and ecosystem effects. Traits in the table include the plant functional traits used in following chapters of this dissertation. Adapted from (Cornelissen 1996, Lavorel and Garnier 2002, Cornelissen *et al.* 2003, Garnier et al. 2007, Quested *et al.* 2007).

	Environmental response	Ecosystem effect
Vegetative traits		
Life form	Reflects strategies and associated traits for avoidance, tolerance and regeneration being a good predictor for responses to disturbance such as fire and grazing.	Associated to primary productivity (carbon stock, resource capture and growth rate), flammability, and decomposition
Canopy height (plant height)	Associated with competitive vigour and response to disturbance	Associated to light capture, fire spread and competition
Leaf dry matter content (LDMC, is the oven-dry mass of a leaf divided by its water-saturated fresh mass)	Are linked to the establishment phase and persistence of plants. Are correlated to relative growth rate and are involved in a trade off between rapid production of biomass and efficient conservation of nutrients. LDMC is also related to resistance to grazing.	Is related to flammability, drought tolerance, growth rate, and decomposition
Specific leaf area (SLA, is the one-sided area of a fresh leaf divided by its oven dry mass)		
Leaf carbon concentration (LNC)		Related to primary productivity (carbon fixation and growth rate)
Leaf nitrogen concentration (LCC)		
Leaf phosphorus concentration (LPC)		
Type of mycorrhiza	Is an indicator of nutrient uptake strategy	Related to primary productivity (resource capture)
Regenerative traits		
Seed mass	Indicator of seed production, dispersal ability and persistence in the seed bank	
Dispersal mode	Indicator dispersal ability	
Onset of flowering	Related to grazing response	

(McIntyre *et al.* 1995, Lavorel *et al.* 1999). Plant traits often linked with grazing response include life history, canopy height plant habit, architecture, or growth from (Lavorel *et al.* 1997, McIntyre *et al.* 1999). However, the history of grazing, as well as site productivity, seem to play an important role in determining what plant traits are consistently associated with grazing (Diaz *et al.* 2007). Results from a meta-analysis of plant response to grazing, based on 197 studies indicated that, overall, grazing favours annual over perennial plants, short plants over tall, prostrate over erect, and stoloniferous and rosette architecture over tussock architecture (Diaz *et al.* 2007). No consistent pattern was found for growth form. Specific leaf area, representing a fundamental trade-off between growth and defence, has also been suggested to be linked with grazing response (Westoby 1999, Cingolani *et al.* 2005). Westoby (1999) suggested that plants with high SLA should be favoured under heavy, non-selective grazing (species with high SLA may be advantaged by faster regrowth) while plants with low SLA should predominate under moderate to low grazing.

Regenerative traits (*e.g.* seed mass, dispersal, persistence in the seed bank) are of great importance for evaluating the response to soil disturbance and fire. It is commonly assumed that the main traits allowing persistence after stand-replacement fires are the resprouting capacity and the ability to form a persistent seed bank (Pausas *et al.* 2004), the latter also important in plant persistence after soil disturbance (Fernández Alés *et al.* 1993, Luzuriaga *et al.* 2005).

Succession may also be seen as a gradient, a complex environmental gradient where the availability of resources is strongly altered (Bazzaz 1996). A general pattern in species traits response to succession seems to be the replacement of fast-growing species (high SLA and LNC, low LDMC) acquiring external resources rapidly, which dominate immediately following abandonment, with slower growing species (low SLA and LNC, high LDMC), which tend to conserve internal resources more efficiently and generally dominate later stages of succession

(Garnier *et al.* 2004). The relation of plant traits with abandonment and succession is approached in Chapter 2.

SPECIES TRAITS AND ECOSYSTEM PROCESSES

Species traits affect ecosystem processes directly through changes in biotic controls and indirectly through changes in abiotic controls (Chapin III *et al.* 2000; Fig. 1). The species traits that are most likely to affect ecosystem processes are those that modify the availability, capture and use of soil resources such as water and nutrients, those that affect the trophic structure within a community, and those that influence the frequency, severity, and extent of disturbances such as fire (Chapin III and Walker 1997).

A well documented mechanism by which species traits affect resource availability is through differences in litter quality, which influences the turnover rate of nutrients in litter and soil organic matter (Hobbie 1992). Litter quality varies markedly between species or species groups (Quested *et al.* 2003). Cornelissen *et al.* (1999) found that slow growing, evergreen, woody species from the British flora differ in litter quality and several leaf traits (lower SLA and palatability) from deciduous woody species and, consequently, decompose slower than the latter. However, this difference was not present in plant species from Mediterranean-type ecosystems where differences in leaf decomposition rate were not consistently different (Gallardo and Merino 1993, Gillon *et al.* 1994). It was suggested that, in ecosystems under relatively strong drought stress, deciduous plants possess protective leaf traits that resemble those of evergreens, even in the more favourable season for growth (Cornelissen *et al.* 1999). Species characteristic from later successional stages, as well as species adapted to low nutrient and/or dry sites, usually produce litter with lower concentrations of nitrogen and phosphorus and with higher concentrations of lignin, tannins, waxes and other recalcitrant compounds that negatively affect microbes and lead to low decomposition rates

(Cornelissen 1996, Gallardo 2000). On the contrary, early successional, as well as high-resource species produce rapidly decomposing litter (Grime 2001, Chapin III 2003).

Another mechanism by which species alter resource supply is through associations between plants and nitrogen-fixing micro-organisms, and mycorrhizal associations between plant roots and fungi (Chapin III *et al.* 2000). Nitrogen fixation and mycorrhizal associations increase the ability of plants to utilize different nutrient pools, thereby enhancing primary production and speeding succession (Read 1994, Klironomos *et al.* 2000, Read and Perez-Moreno 2003).

Grazing has a major impact on ecosystem processes because it influences the quantity and quality of resources that become available for decomposers (Wardle and Bardgett 2004). Herbivores transfer plant tissue to the soil before nutrient resorption can occur, they select preferentially nutrient-rich tissues, and they excrete nutrients in readily available form, short-circuiting the decomposition process (Hobbie 1992, Bardgett 2005). Plant traits related to protection against herbivores, such as physical leaf toughness, physical barriers (*e.g.* wax, spines, hairs) and secondary compounds also affect soil microorganisms resulting in slower decomposition rates (Cornelissen 1996, Cornelissen *et al.* 2003).

Some traits of plant species strongly influence the probability or severity of many disturbances (Chapin III 2003). Fire is a common disturbance in Mediterranean vegetation and important traits linked with this kind of disturbance include those that relate to flammability and fire spread (Lavorel and Garnier 2002). Traits influencing flammability include those that determine tissue moisture, such as water content, and those conferring drought resistance (Table 1). Fire spread is related to the total biomass accumulation and its spatial arrangement, which are linked to traits such as canopy height and structure (Table 1, Cornelissen *et al.* 2003).

Other ways in which plant species affect their environment include effects on soil structure and chemical properties, which in turn influence hydrology, plant growth, biogeochemical cycling, and the activity of soil organisms (Eviner and Chapin 2003). Plant species can influence microclimate by influencing surface temperature, humidity, water loss and light (Moro *et al.* 1997, Berendse 1998). A study in an annual grassland of California showed that different plant species differed in their effects on soil labile carbon, soil temperature, and soil moisture, which are chemical soil properties with great importance in ecosystem functioning (Eviner 2004). Species effects on soil temperature were most pronounced during the cold winters, when temperature is limiting plant and microbial activity, while species effects on soil moisture become stronger in the warm, dry spring (Eviner 2004). The chemical properties of plant litter and exudates influence many chemical properties of the soils, such as pH and nutrient content (Berendse 1998, Van Breemen and Finzi 1998, Knops *et al.* 2002).

Biomass production

Biomass production and accumulation integrate plant responses to biotic and abiotic features of their environment. Plant biomass may be used to estimate primary production, nutrient pools, species dominance, responses to experimental manipulations or fuel loads for fire (Northup *et al.* 2005).

Net primary productivity (NPP) is the amount of carbon and energy that enters ecosystems and represents the net carbon gain over a particular time period (usually a year). It is the best summary variable of ecosystem processes, being the result of numerous interactions among elements, organisms and environment, and it can be considered as an integrative variable of the functioning of the whole ecosystem (McNaughton *et al.* 1989).

Net primary productivity is influenced by the resources available and by environmental conditions, and the relative importance of these factors varies with scale and ecosystem (Chapin III and Eviner 2003). At the global scale, water is the most limiting resource of above-ground primary productivity (ANPP) and changes in ANPP are often related to mean annual precipitation (Jobbágy and Sala 2000). At regional or local scales the variation in primary productivity depends on soil resources and disturbance regime (Díaz *et al.* 2005).

Biomass and net primary productivity also vary among species and life forms. Differences result from a wide range of plant traits, including growth rate, allocation patterns, phenology, nutrient use efficiency, resource requirements, traits that influence assess to resource pools (*e.g.* root depth or symbiosis with mycorrhiza or N-fixing microorganisms), and traits that influence conditions that limit growth (*e.g.* temperature and moisture, Chapin III and Eviner 2003). Therefore, changes in structure and composition of vegetation are often accompanied by changes in biomass and net primary productivity (Chapin III *et al.* 2002).

Litter decomposition – a key process

Decomposition is a key process in the functioning of ecosystems enabling the recycling of chemical elements (Chapin *et al.* 2002). Through decomposition the fixed carbon incorporated into above and belowground biomass returns to the atmosphere as CO_2 and is incorporated into the soil as stable soil organic matter (Schulze *et al.* 2002). Decomposition also means the return of nutrients, other than carbon, to mineral form which can be used by plants for biomass production (Berg and Laskowski 2006b). The rate at which decomposition occurs determines the availability of these nutrients to plant and therefore influences primary production (Berg and Laskowski 2006b).

The pattern of litter mass loss varies during the decomposition process and three main phases can be identified (Chapin 2002). During the first phase leaching of the cell solubles is

the predominant process; therefore initially there is a rapid decrease in litter weight due to the loss of rapidly metabolizable compounds or simply readily leachable substances (Fig. 2). The second phase involves a combination of fragmentation by soil animals, chemical alteration by soil microbes and leaching of decay products from litter. The second phase is controlled by litter quality. Litter with lower concentrations of nitrogen and phosphorus and with higher concentrations of lignin, tannins, waxes and other recalcitrant compounds that negatively affect microbes lead to low decomposition rates (Berg 2000). The final phase occurs quite slowly and involves chemical alteration of organic matter that is mixed with mineral soil and leaching of breakdown products to other soil layers and is often regulated by lignin decomposition (Berg and Laskowski 2006a).

Litter decomposition is controlled by abiotic factors such as climate and soil chemical and physical properties and by biotic factors such as litter quality and the nature and abundance of the decomposer organisms (Coûteaux *et al.* 1995, Aerts 1997). Climate is known to influence the biodiversity of microbial and animal communities, as well as the duration and the intensity of their activity, through its effects on temperature and soil moisture (Coûteaux *et al.* 1995). Temperature affects decomposition directly by promoting microbial activity and indirectly by altering soil moisture (decomposers are more productive under warm moist conditions), and the quantity and quality of organic matter input to the soil (Chapin III *et al.* 2002). Consequently, climate strongly determines decomposition rate, particularly in Mediterranean ecosystems where there is a temporal asynchrony of favourable temperature and moisture conditions (Moro and Domingo 2000). Mediterranean ecosystems benefit from pulses of moisture, which can speed decomposition, but in contrast the soil surface tends to frequently dry rapidly during the periods of favourable moisture and temperature conditions (autumn and spring), thereby preventing the formation of stable communities of soil micro-organisms and slowing down decomposition (Gallardo 2000).
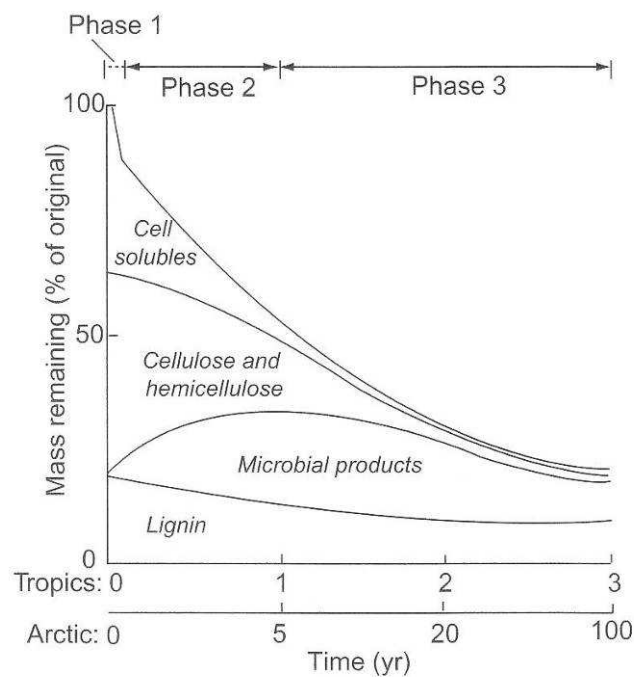
Figure 2 - Representation of the three major phases of litter decomposition, and time scales for leaf-litters from two contrasting environments (warm tropic and cold tundra). Leaching dominates the first phase of decomposition. Substrate composition changes during decomposition because labile substrates, such as cell solubles, are broken down more quickly than are recalcitrant compounds, such as lignin and microbial cell walls. Figure taken from (Chapin III *et al.* 2002)

Although climate is the factor that most strongly influences decomposition, within a particular climatic region, litter quality is the factor exerting the strongest control on litter decomposability (Aerts and Chapin 2000). A large number of studies assessed the relationship between several litter chemical and physical parameters (such as foliar nutrients, components of leaf structure and plant defences), and decomposition rates in different ecosystems (*e.g.* (Gallardo and Merino 1993, Gillon *et al.* 1994, Berg 2000, Pérez-Harguindeguy *et al.* 2000, Wardle *et al.* 2002, Aerts *et al.* 2003, Quested *et al.* 2007). In general terms, fast growing (high foliar nutrient contents), poorly defended species, which dominate early stages of succession, produce litter with high decomposition rates while slower growing, better defended species, which are common in later stages of succession, produce poor-quality litter with low

decomposition rates (Cornelissen *et al.* 1999, Grime 2001). The relationship between litter quality and decomposition with secondary succession is further discussed in Chapter 4.

SECONDARY SUCCESSION

After disturbance, ecosystems undergo succession, which can be defined as a reasonably directional and predictable change in communities resulting from biotically driven changes of the physical environment (Odum 1969). In the absence of disturbances, it culminates in a stabilized ecosystem in which the developed community is in equilibrium with the prevailing environment (Odum 1969, Noble and Slatyer 1980). Secondary succession, in opposition to primary succession, which occurs in an area originally devoid of life, generally begins with a more mature soil containing a sizable bank of seeds and vegetative propagules (Crawley 1997, Chapin 2002). The type of disturbance initiating secondary succession influences the successional pattern, particularly in the case of managed areas, where the past land uses have an important influence on the vegetation dynamics and may lead to different successional patterns (Bonet 2004).

Different theories exist to explain the adaptation of plants and mechanisms of successional change. The model of species replacement states that plant species modify their environment creating conditions that favour the establishment of other species but becomes less suitable for its own persistence (Clements 1936, Correia 1998). According to other authors, species from different stages of succession are present at a site at the beginning of succession and it is this initial species composition that determines the future shifts in dominance. This theory suggests that there is a gradual replacement of species based on their longevity rather then a substitution of one group of species by another (Egler 1954 in Noble and Slatyer 1980). More recently Connell and Slatyer (1977) proposed three main types of pathways to explain successional sequences: (1) Facilitation pathway, where species change their environment so

that it favours the establishment of other species; (2) Tolerance pathway, in which later successional species become established and grow in the presence of earlier species because they can grow at lower light and nutrient availability; (3) Inhibition pathway, which assumes that early successional species inhibit the establishment and growth of species from later successional stages (Noble and Slatyer 1980). By the same time Grime (1977) proposed a three-strategy model based on primary strategies – competititors, stress tolerators and ruderals (CSR). This theory derives from a two-dimensional ordination of the favourability of the environment and the level of disturbance, in which one of the extremes (unfavourable, disturbed sites) is uninhabitable, leaving a triangle. Competitors occur in conditions of low stress and low disturbance. Stress tolerators occur in unfavourable but undisturbed sites were the resistance to environmental extremes is an important selective force. Ruderals occur in environments that are favourable but disturbed and that, therefore, offer little competition for resources (Grime 2001). Tilman (1982 in Bazzaz 1996) explains succession in terms of availability of resources, especially nutrients and light. He assumes that throughout succession there is an inverse gradient of nutrient and light availability, i.e., an area changes with succession from low-nutrient and high-light environment to high-nutrient and low-light environment. The competition for limiting resources is important in the determination of the dominant species. In this model it is assumed that competing species experience trade offs in their resource requirements such that a superior competitor for one resource is an inferior competitor for other resources (Tilman 1990). To cope with succession of agricultural fields, Gleason & Tilman (1990) proposed the transient dynamics hypothesis. Succession is the result of dynamics where the species of early stages of succession having a strong colonization ability and/ or fast growth rate are progressively replaced by species with a strong root biomass and/ or low tissue nitrogen content (Gleason and Tilman 1990).

Nutrient and vegetation dynamics along the succession gradient

Nutrient dynamics in ecosystems is governed by the balance between supply from the soil and demand by the plants and it is generally agreed that the availability of soil nutrients changes during ecological succession (Vitousek *et al.* 1989, Bazzaz 1996). The most dramatic changes in the soil occur in the first year of succession and the major factors in these changes are the addition of organic matter, the development of a litter layer and the stabilization of the soil surface (Bazzaz 1996). Initial soil characteristics such as fertility, temperature and moisture, and the nature of disturbance that initiates the succession are important determinants of nutrients dynamics during succession (Foster *et al.* 2003 Chapin 2002). The disturbances that initiate secondary succession generally cause increased nitrogen availability in the disturbed site because the removal of the dominant vegetation increases soil temperature and moisture causing an increase in decomposition and nitrogen release (Vitousek *et al.* 1989). Additionally the initial colonists in secondary succession are often fast-growing species with high nutrient contents and relatively high rates of decomposition and nutrient release (Grime 2001). After this initial pulse nitrogen is expected to decline. The pattern of changes during succession of old-fields, however, is less clear and may depend on the duration and intensity of agricultural use (Vitousek *et al.* 1989). The availability of phosphorus, in contrast, usually declines with community age, as soil phosphorus is lost by erosion, chemical inactivation and transfer to intractable organic complexes (Fitter and Hay 1987).

During secondary succession there are various developments, which include increasing modifications of the soil and micro-climate by the vegetation, increasing interaction between plants, declining frequency of seedling establishment, and increasing stratification of the vegetation (Bazzaz 1996). Early successional species are usually short-lived and typically have high relative growth rates, supported by high rates of photosynthesis and nutrient uptake. These species reproduce at an early age, allocate a large proportion of net primary productivity

to reproduction and usually have good dispersal ability (Grime 2001). As succession proceeds there is a gradual shift in dominance to species that have lower resource requirements and grow more slowly (Rees *et al.* 2001).

The main factors determining the species present in different stages of succession include colonization, initial floristic composition, life history traits and interactions between species (Neeman and Izhaki 1996). The ability of species to colonize new sites is related to several regeneration traits, such as seed dispersal, seed germination, seed mass and seedling competition and establishment (Chapin *et al.* 1994). The changes in species composition that occur after the initial colonization of a site result from a combination of life history traits of colonizers, facilitation and competitive interactions, herbivory and stochastic variation in the environment (Noble and Slatyer 1980, Bazzaz 1996).

Facilitation involves processes in which early successional species make the environment more favourable for the growth of later successional species (Chapin III *et al.* 2002), and has been referred to be important in harsh environments (Pugnaire *et al.* 1996). Mechanisms of facilitation include microclimate amelioration, increased water availability, and improved soil fertility, and nitrogen fixation and mycorrhizal associations (Rodriguez-Echeverria and Pérez-Fernández 2003, Maestre *et al.* 2005, Callaway and Pugnaire 2007). Nitrogen-fixing associations are commonly found in developing communities, at middle stages of succession. As N concentration in the soil increases, the benefits to the fixing species decline and the cost of symbiosis presumably remain, rendering the species less competitive (Fitter and Hay 1987). In contrast, and because phosphorus usually declines with succession, the benefits of having mycorrhiza would be expected to be an advantage and to increase as the community matures (Schulze *et al.* 2002). Short-lived plants that colonize early stages of succession are the ones most commonly found to be non-mycorrhizal (Read 1994).

Competitive interactions include competition among neighbours for limited resources and competition by chemical interference (Crawley 1997). Competition for resources such as light, water, nutrients and space are important in plant communities and become intense during succession as vegetation develops and biomass increases (Bazzaz 1996). Competition by chemical interference, *i.e.*, allopathic phenomena, are an important factor influencing community composition and structure, particularly in Mediterranean ecosystems where harsher climatic conditions seem to increase the production of allelopathic compounds (Herranz *et al.* 2006). Allelopathic phenomena have been reported for some Mediterranean shrubs, in particular for species belonging to the Cistaceae family (Chaves and Escudero 1997)

The importance of herbivory in ecosystem function has been approached in previous sections. Regarding the role of herbivores in succession, it differs among ecosystems and successional stage (Chapin III *et al.* 2002). In grasslands, herbivores prevent the establishment of woody species that might otherwise transform grasslands into shrubland and forests (Chapin III *et al.* 2002) thereby slowing down succession.

FROM INDIVIDUAL PLANTS TO COMMUNITIES OR ECOSYSTEMS – THEORY AND RESULTS FROM EMPIRICAL STUDIES

It is in linking vegetation structure and ecosystem processes where the use of plant functional traits provides the greatest contribution (Díaz *et al.* 1999). A framework to link plant traits and ecosystem processes was proposed by Chapin III *et al.* (2000). This framework was further refined by Lavorel and Garnier (2002) and it proposes that environmental changes will lead to changes in community composition and thus in plant traits, and these in turn will affect ecosystem functioning (Fig. 1). This is based on the hypothesis that plant traits can simultaneously explain individual plant responses to environmental changes, and effects on

ecosystem processes (Lavorel and Garnier 2002). The overlap between response and effect traits (Table 1) seems to be strong for traits related with the resource axis (*e.g.* SLA, LNC, LDMC) and week for regeneration traits associated with response to disturbance (Lavorel *et al.* 2007).

An approach used to link species effect traits to ecosystems processes is based on the effect of dominants, known as the biomass ratio hypothesis (Grime 1998). The biomass ratio hypothesis states that the extent to which a plant species affects ecosystem functions is likely to be closely predictable from its contribution to the total biomass. Community-aggregated traits, which are the average community value for a trait weighed by species abundance, allow the combination of information on community structure and plant traits, and they can be used to relate plant traits to ecosystem functioning or to detect the average functional response of vegetation to environmental factors (Violle *et al.* 2007). When the community-aggregated traits are calculated for variable traits, *i.e.* for traits differing in value according to treatment levels, then a change in their values can be caused either by variability of traits within species, by a change in species composition, or both (Garnier *et al.* 2004). For the test of the mass ratio hypothesis of ecosystem functioning the change in aggregated traits is probably the most important community characteristic (Garnier *et al.* 2007). Such a test was conducted for the project Vista (box 2) site in Southern France by Garnier *et al.* (2004). The authors showed that community-aggregated SLA, LDMC and LNC were correlated with specific above-ground net primary productivity (SANPP – expresses productivity on a community basis instead of ground area basis), but relationships between these traits and biomass and ANPP where not significant.

Box 2. Project VISTA – Vulnerability of ecosystem services to land use change in traditional agricultural landscapes (2003-2005)

The project aimed at compiling an integrated assessment of the vulnerability of European traditional agro-pastoral landscapes to land use change that would assist land managers and regional policy makers for the prioritisation of sustainable development strategies. The project used the conceptual framework of Plant Functional Traits (PFT) to predict the dynamics of, and the services provided by, traditional agro-pastoral landscapes and applied a new scenario-based approach to integrate ecological, socio-cultural and economic data in order to represent current and potential future trends, shaped by global and regional contexts. The project involved 11 sites, in marginal agricultural areas, from 9 countries.

The present work was mostly carried out under the framework of the work package named "Linking plant traits to population and ecosystem functioning". The main objectives of this work package were:

1. Based on a core list traits proposed by Weiher (1998) aiming at an understanding of vegetation, to test whether traits selected ("soft" traits) are reliable surrogates for the underlying functions and processes ("hard" traits) they are supposed to capture. These relate to patterns of resource acquisition and use, competitive ability and plant demography

2. To test, using a network of 11 sites, how some of these soft traits vary along gradients of land use change, identifying the direct underlying gradients in each case

3. To identify relationships between species traits and ecosystem processes

4. To establish synthetic indices of ecosystem functioning and indicators that can be used to assess and predict impacts of land-use changes on ecosystem services

5. To feed data bases of plant traits for Western and Mediterranean Europe

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STRUCTURE AND OBJECTIVES

In the context of present land use changes, this work aims to contribute to a better understanding of the effect of abandonment on ecosystems with a long history of human management, such as the extensive agro-pastoral systems of South Portugal. Abandonment is a major factor leading to secondary succession in these areas, and its consequences are still little understood. These land use changes are followed by alterations in the soil characteristics

and vegetation composition and structure. The species present at a site, as well as those that colonize throughout succession, have a set of functional traits that allow them to colonize and persist, that determine their response to environmental factors, and that will determine the change in species richness and composition throughout succession (Fig. 3). The species that colonize different stages of succession likely differ in plant functional traits that influence rates of biomass accumulation and quality of the litter produced with consequences for ecosystem processes, such as above-ground net primary productivity and litter decomposition.
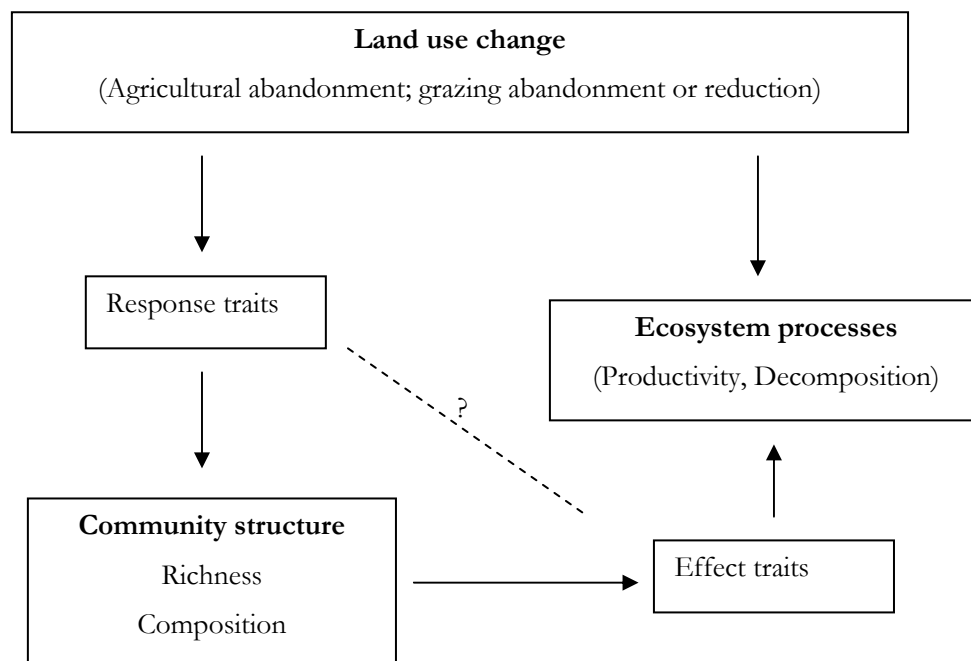
Figure 3 - Land use change contributes to changing species diversity in term of richness, composition as a result of changes in species response traits. Changes in species effect traits affect ecosystem processes (adapted from Chapin III *et al.* 2000, Lavorel and Garnier 2002). As discussed above, response and effect traits may or may not coincide.

The objectives of this thesis were to:

1. Identify changes in vegetation composition and structure in response to decreasing land use intensity (Chapters 1 and 2)

2. Identify functional groups and changes in species traits in response to decreasing land use intensity (Chapter 2)

3. Identify the effect of decreasing land use intensity in key ecosystem processes such as decomposition and above-ground biomass and net primary productivity (Chapters 3 and 4).

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CHAPTER 1

SITE CHARACTERIZATION: VEGETATION COMPOSITION AND SOIL PROPERTIES

INTRODUCTION

Current and historical human activity is an unavoidable subject when studying the structure and function of ecosystems because it strongly influences modern vegetation patterns (Foster *et al.* 2003).

The South of Portugal, and in particular the region of Baixo Alentejo, has a long history of human activities that date from pre-roman times (Serrão 1963) and that lead to great transformations in the vegetation. The original vegetation, thought to be formed by holm oak woods mixed with other species with persistent leaves (such as *Quercus suber*, *Olea europaea* var. *sylvestris*) has been transformed throughout history by human activities (Onofre 2007). These are thought to date from Palaeolithic times and to have intensified through time with increases in cultivated areas and with the demand of wood for naval construction in the XV and XVI centuries (Capelo and Catry 2007). A review by Roxo *et al.* (1998) describing the history of land use in the municipality of Mértola indicates that, in the beginning of the 18th century, most of the area was covered with montado and matorral that were used as commons for hunting, collecting of honey and firewood, and grazing (sheep, pigs and goats). A significant increase in the population of the municipality at the end of the 18th century followed by policies focusing on the enlargement of agricultural production at the end of the 19th century resulted in the enlargement of the areas used for cereal growth and pasture (Roxo *et al.* 1998). In addition, the wheat campaign, which lasted from 1926 until the late 60's, strongly contributed to the transformation of vegetation and landscape in this part of the country (Roxo *et al.* 1998, Capelo and Catry 2007). This long history of human occupation and activity has been followed, since the last decades of the 20th century, by a decline in land use intensity (Pinto-Correia and Vos 2004).

The colonization of recently disturbed areas with annuals and short-lived species and their subsequent replacement with perennials, tall shrubs, and trees during post-disturbance

succession has been frequently reported (*e.g.* Houssard *et al.* 1980, Debussche *et al.* 1996, De Bello *et al.* 2005). However, the types and duration of disturbances strongly affect vegetation dynamics following abandonment (Chapin III *et al.* 2002, Bonet 2004, Peco *et al.* 2005). Secondary succession after disturbance is determined both by the pre-disturbance community and by the input of propagules from the surrounding landscape (Tatoni and Roche 1994, Pugnaire *et al.* 2006). Centuries of agricultural practices may have limited the availability of propagules, thus influencing the rate and pattern of secondary succession after abandonment (Lavorel *et al.* 1999, Bonet 2004).

The imprints of past land use on soil properties, likely caused by agriculture, burning and grazing may also have important consequences for the function and dynamics of ecosystems (Foster *et al.* 2003). Agricultural practices cause a decrease in soil carbon and nitrogen due to a decreased plant organic inputs and to increased decomposition and erosion (Knops and Tilman 2000, Post and Kwon 2000) Additionally, soil properties are known to change during secondary succession (Vitousek *et al.* 1989, Bautista-Cruz and del Castillo 2005). Some authors report changes in soil carbon (Knops and Tilman 2000, Post and Kwon 2000), nitrogen (Knops and Tilman 2000) and phosphorus (Ruecker *et al.* 1998) during secondary succession. Soil carbon and nitrogen content, and organic matter increased with age of abandonment while phosphorus decreased, in old-field in southern France (Escarré *et al.* 1983).

The main objective of this chapter is to present a characterization of the study site. First we present a short summary of recent land use and land cover changes, which have served as basis for the selection of the study site and for the different land use categories within the site. Second, we look at soil physical and chemical properties and vegetation composition within each land use category.

STUDY SITE

The study site (Monte do vento, Mértola) is located in the Southeast of Portugal (Fig. 1), at about 37°48'21.72" N and 7°40'44.96" W.
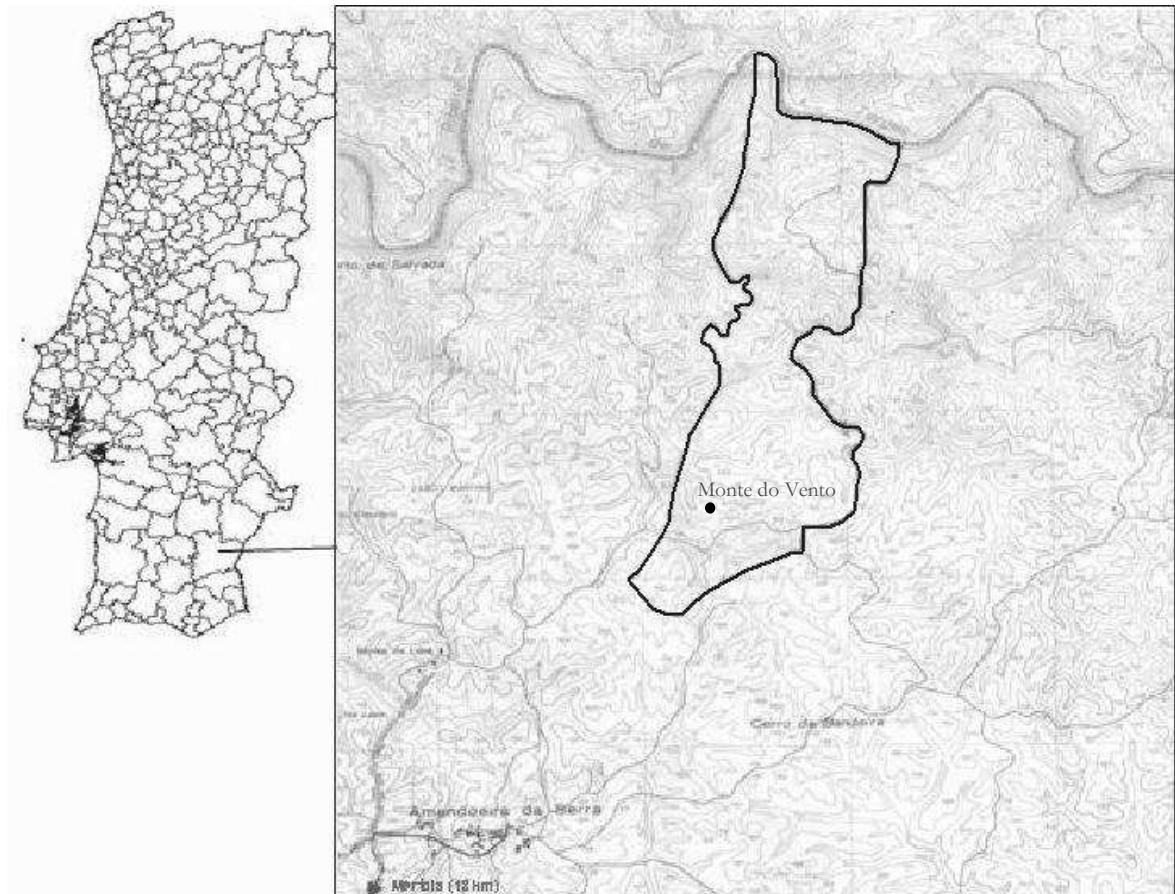
Figure 1 -. Location of the study site. Map of Portugal (taken from Atlas do Ambiente) and part of the military map nº 541 (Amendoeira da Serra). ▭ - Limits of the study site (Monte do Vento).

The climate is Mediterranean with mean annual temperature of 16.8° C and mean annual rainfall around 438 mm (Fig. 2), with large inter-annual fluctuation. Most of the precipitation is concentrated during the autumn and winter months. Summers are dry and hot. The main bedrock is schist and the type of soil occurring in the study site is a poor and shallow lithosol of non-calcareous schist (Roxo *et al.* 1998). Soils are characterized by stony, dominantly

mineral, often thin horizons that merge into or rest on shattered hard rock at no great depth below the surface. Their effective depth is normally close to 10 cm (Cardoso 1965).
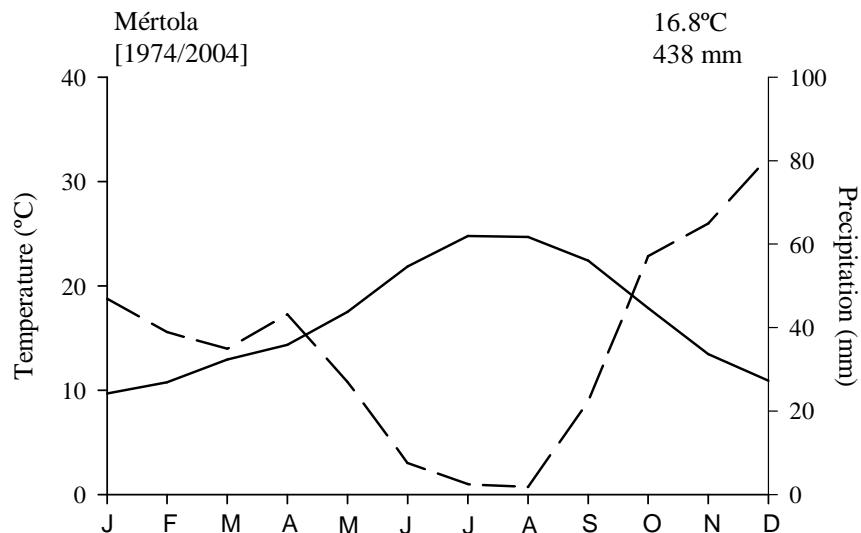
Figure 2 - Climatic diagram of Mértola from 1974 to 2004 (data from the Instituto Nacional de Meteorologia, Portugal).

Land use

A recent assessment of changes in land use and land cover from 1958 to 2000 in Amendoeira da Serra (area of the study corresponds the military map nº 541, Amendoeira da Serra), where the study site is inserted, was done by Van Doorn (2007). As a consequence of the wheat campaign, in 1958 the landscape was very homogeneous, dominated by arable / grassland (66%) almost without tree cover. The areas of high dense matorral were limited to the steep river banks and the area occupied by montado was small (16%). The trend in land cover change that occurred in the following decades reflects an extensification in cultivation, with declining activities like ploughing and other types of soil tillage. There was a decline in the area occupied by arable / grassland and a gradual increase in the areas occupied by montado and matorral. Forestation programs for less favoured areas as part of the second

pillar measures of the CAP favoured the emergence of forest plantations (occupying an area of 30 % in 2000). At present, the land use is dominated by extensive farming systems and the main land use activities are livestock (mainly sheep) raising and some cereal production, mainly for fodder. Some land owners practice a rotation cycle, generally of 3 years with one year of cereal growing and 2 years of fallow in combination with grazing while others only have livestock. There are also land owners that use their land for hunting reserves or new forest plantations (often holm or cork oak but mixed or pine plantations are also found). This extensive use of soil in combination with heterogeneous physical conditions has resulted in various densities of shrub cover, representing different degrees of the intensity and type of management.

Within the area surveyed by the above mentioned study, a site (Monte do Vento) was selected where different land uses and land cover types coexist.

METHODS

Experimental design

At the study site, three land use categories were selected to represent a decrease in land use intensity (Fig. 3). Common to the three land use categories is past land use, which consisted of rotation of crops / fallow / pasture. The first category, 'grazing' (Fig. 4), corresponds to areas of pasture where cropping stopped more recently (about 7 years before the beginning of this study in 2003). These are grasslands with disperse cork and holm trees, and are used for extensive grazing by sheep (0.99 CU/ha). The herd visits the area twice a year, at the end of winter and beginning of summer. The two other categories, 'intermediate succession' and

'advanced succession'[1] correspond to abandoned areas. 'Intermediate succession' plots are abandoned for 10 to 15 years (Fig. 5). 'Advanced succession' plots are abandoned for 20 or more years (Fig. 6) and likely correspond to areas where soils first showed signs of degradation or stoniness too high for agricultural machinery.

Land cover

◻ Forest plantation
◻ Low, scattered shrub, 20-50% shrub cover
◻ Middle, discontinuous shrub, 50-75% cover
◻ Pasture/arable <20% shrub cover
◻ Urban area
◻ Water lines/reservoirs

Land use category

● Grazing
■ Intermediate succession
▲ Advanced succession
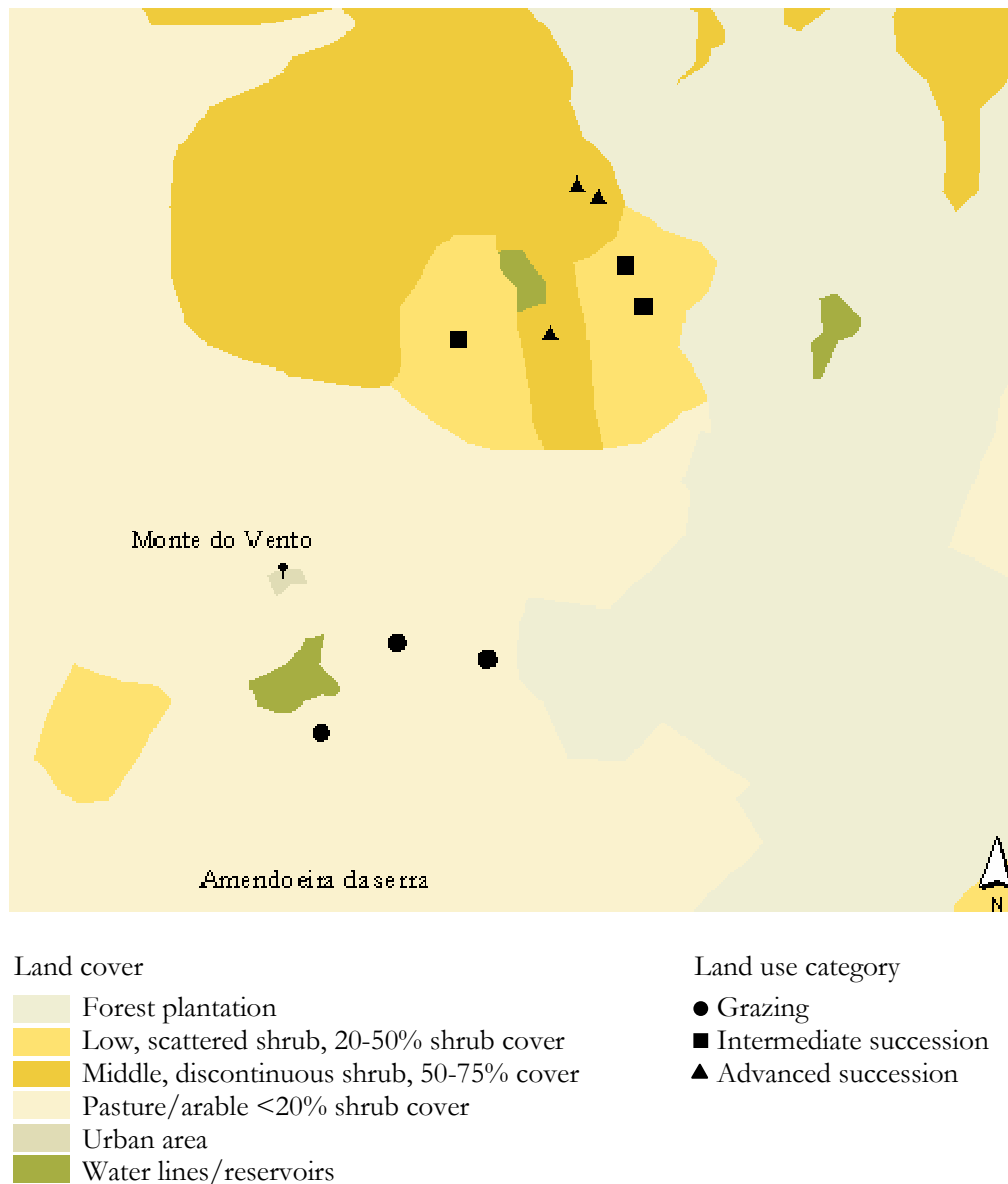
Figure 3 - Main land cover types in the study site and land use categories selected for this study, scale 1:4000. Map and land cover types adapted from (Van Doorn 2007). Symbols represent the position of the three plots within each land use category.

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[1] The names of land use categories were chosen to fit existing categories from project VISTA. We do not intend to discuss whether category names are appropriate considering the time of abandonment.

Figure 4 - General view of 'grazing' land use category.

Figure 5 - General view of 'intermediate succession' land use category.

Selection and estimation of time since abandonment of the land use categories were based on aerial photographs and land cover maps (Van Doorn 2007), personal interviews to landowners and management plans or reports from ADPM (Associação de Defesa do Património de Mértola). Abandonment refers only to ploughing, as no data was available for cessation of grazing.

Within each land use category, three permanent plots where set. Plot size was adapted to the type of vegetation resulting in plots with 900 m^2 in 'grazing' and 2500 m^2 in 'intermediate' and 'advanced succession'.

Figure 6 - General view of 'advanced succession' land use category

Data collection

Vegetation sampling

Vegetation composition was obtained by recording species presence and percent cover in 15 quadrats randomly distributed within each plot of the three land use categories. The size of the quadrats was scaled to the size of the occurring type of vegetation resulting in quadrates of 0.25 m^2, 2 m^2, and 4 m^2, in the land use categories 'grazing', 'intermediate succession', and 'advanced succession', respectively (Kent and Coker 1992). Species nomenclature follows Tutin *et al.* (1964-1980). Percent cover of each species, inside each plot was then obtained as the mean value of the 15 quadrats. In addition, five *a priory* groups (annual herbs, perennial herbs, small shrubs, tall shrubs and trees) were identified.

Soil sampling and analysis

The methodology used for soil sampling and analysis is described in Garnier *et al.* (2007). Soil samples (0-5 cm horizon) were collected at the end of the winter period. In each plot, twelve cores, distributed on the whole plot area, were collected and bulked to make a composite sample representative of the plot. Prior to analysis, soils were crumbled by hand,

dried for one week at room temperature and then sieved through a 2mm mesh. The following variables were measured: pHwater, texture (sand, loam and clay), total carbon and nitrogen concentrations, and available phosphorus (Olsen procedure). The soil analyses were done by the "Laboratoire d'Analyses des sols" of the National Institute for Agronomic research (INRA, Arras, France). The water holding capacity was derived from the texture data, using a modified version of the equation provided by Saxton et al. (1986; see http://www.bsyse.wsu.edu/saxton/).

Data analysis

Significant differences in soil parameters among land use categories were tested with one-way analysis of variance on untransformed data. Mean differences were separated with Tukey's HSD test at 5% level of significance. All statistical analyses were performed with SPSS 14.0 for windows (SPSS, Inc. Chicago, IL).

RESULTS AND DISCUSSION

Vegetation composition

A total of 99 species belonging to 24 botanical families and 71 genera were recorded (Appendix 1). The most numerous family was Leguminosae with 21 species (21.21 %), followed by Compositae (17.17 %), Gramineae (16.16 %), Cistaceae (6.06 %), and Caryophyllaceae (6.06%), together representing 66.66 % of the total number of species.

'Grazing' land use category was mostly composed of annual species (Fig. 7, Appendix 1), representing 97.35% of plant cover, in accordance to what has been reported for many Mediterranean pastures (*e.g* Peco 1989, Lavorel *et al.* 1999, Azcarate *et al.* 2002). The most

abundant species include forbs with basal rosette or prostrate growth which is seen as an adaptation to grazing (Noy-Meir *et al.* 1989).

'Intermediate succession' land use category was composed of a mixture of herbaceous species, mostly annuals, and shrub species (Appendix 1), representing 33.52% and 65.1% of plant cover, respectively. 'Advanced succession' plots were composed of tall, more or less, dense shrubs (Fig. 7), where the most abundant species was *Cistus ladanifer* (Appendix 1).

The tree species found were often a combination of *Quercus rotundifolia* and *Quercus suber*, and usually scarce and disperse (Appendix 1).
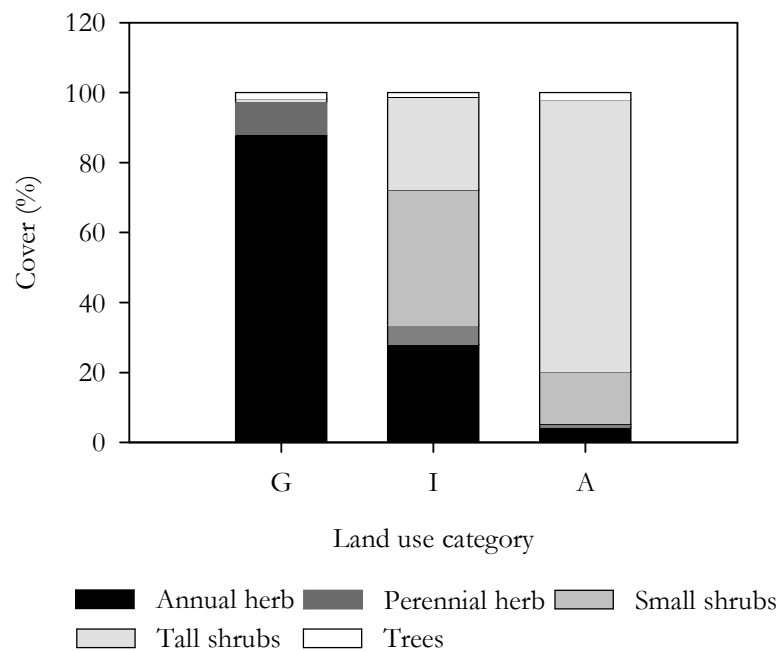
Figure 7 - Change in relative cover of different *a priori* groups after abandonment. Land use categories: G – grazing, I – Intermediate succession, A – advanced succession.

Similarly to many studies in Mediterranean old-fields (*e.g.* Houssard *et al.* 1980, Escarré *et al.* 1983, Debussche *et al.* 1996, Bonet and Pausas 2004) in the first years after cessation of agriculture the vegetation is dominated by annual species. The results from this study differ

from other studies in Mediterranean areas in the lower number and cover of perennial herbaceous and the lower amount of time needed to reach a high cover of woody species. In the present study, woody species accounted for about 65 % of plant cover after a decade of abandonment. This pattern can be attributed to an early colonization of the shrub species facilitated by their life history traits and to the availability of seeds. The dominant shrub species found in intermediate and advanced succession were also present in grazing land use category but with very low percent cover (Appendix 1).

Soil characterization along the succession gradient

Soil texture results indicated that all land use categories have sandy loam soils and statistical analyses of soil texture data did not show significant differences in clay (F=0.467, p>0.05), silt (F=0.352, p>0.05), and sand (F=0.404, p>0.05) content between land use categories.

Soil water holding capacity, pH and soil nitrogen content did not differ significantly among land use categories but phosphorous significantly decreased, while the C:N ratio significantly increased with time of abandonment (Table 1).

A decrease in phosphorous after cessation of agricultural practices, as well as, higher values in grazed grasslands compared to ungrazed scrublands was reported in a study from another Mediterranean area (Ruecker *et al.* 1998). Higher values of phosphorus in grazed areas may be related to grazing and to the subsequent return of phosphorus in faeces (Ruecker *et al.* 1998). Other causes may also be possible. On the one hand, larger fertilizer load in more recently abandoned plots may contribute to higher values of phosphorus in these plots, and in contrast absorption by plants or immobilization in the soil (Escarré *et al.* 1983) may contribute to the decrease of phosphorus with abandonment time.

Table 1 - Soil nitrogen (N), carbon (C), phosphorus (P), carbon to nitrogen ratio (C:N) and water holding capacity (WHC) in the three land use categories. Values are means ± 1 s.e. (n=3). The results of the one-way ANOVA are presented in the last row (* p < 0.05). Values with different superscript letter were significantly different (significance of Tukey-test given at p<0.05).

	N (mg g-1)	C (mg g-1)	P (mg g-1)	C:N	Organic matter (mg g-1)	WHC (%)	pH
G	1.44± 0.14	12.73± 1.61	0.010± 0.001(a)	8.81± 0.39(b)	21.96± 2.79	0.23± 0.003	5.48± 0.02
I	1.56± 0.01	13.87± 0.43	0.008± 0.0006(a,b)	8.87± 0.28(b)	23.92± 0.73	0.23± 0.01	5.63± 0.13
A	1.48± 0.12	15.93± 1.07	0.006± 0.0004 (b)	10.84± 0.55(a)	27.49± 1.85	0.22± 0.004	5.56± 0.11
One-way Anova							
F	0.355	2.006	7.008*	7.547*	2.006	0.426	0.597

Soil nitrogen was not significantly different nor did it show any trend following abandonment. Although some studies carried out in Mediterranean areas have shown an increase in soil nitrogen after abandonment (*e.g.* Escarré *et al.* 1983, Peco *et al.* 2006) it has also been suggested that succession following a period of chronic disturbance (*i.e.* prolonged agricultural use) does not follow such clear patterns; the duration and intensity of disturbance may control whether nitrogen availability and potential nitrification increase or decrease early in such seres (Vitousek *et al.* 1989).

Soil carbon and organic matter were also not significantly different between land use categories, which is due to the outlier values obtained for one of the grazed plots. These two variables showed an increasing trend with abandonment time (Table 1) and, if we remove the outlier plot, soil carbon and organic matter content are significantly higher in 'advanced succession' than in 'grazing' (F=8.01, P=0.023). Increases in soil organic matter and carbon content were found in other old-field studies in Mediterranean areas (Escarré *et al.* 1983) and

non-Mediterranean areas (Knops and Tilman 2000). Soil organic matter pools and carbon availability are controlled by the formation of organic matter through primary production and its loss through decomposition (Zak *et al.* 1990). Grazed plots are dominated by annual plants, which generally have lower content of carbon and C:N ratios, and have higher rates of decomposition than the woody species (Cornelissen *et al.* 1999, Dorrepaal *et al.* 2005) that dominate abandoned plots. Additionally, grazed plots likely have lower organic matter inputs to the soil because part of the production is consumed by herbivores. Moreover, the retranslocation of nutrients from senescing leaves prior to abscission is highly developed in *Cistus ladanifer* for nitrogen and phosphorus, as is often the case in Mediterranean shrubs that colonize soils of low fertility (Núñez-Olivera *et al.* 1993, Fioretto *et al.* 2003). The increase in shrub cover with abandonment time may therefore result in the input of litter that has higher C:N ratio and is poorer in phosphorus than that resulting from herbaceous species, contributing to higher values of soil C:N and carbon and to lower values of phosphorus in 'advanced succession' plots.

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CHAPTER 2

FUNCTIONAL RESPONSE AND EFFECT TRAITS IN RELATION TO LAND USE CHANGE IN EXTENSIVE AGRO-PASTOTAL SYSTEMS

INTRODUCTION

Vegetation change has traditionally been described by changes in species composition. However, the need to predict the effects of climate and land use change on vegetation structure and ecosystem functioning at regional and global scale has lead to an ongoing effort to identify plant functional traits and types that relate to climate and land use change (McIntyre *et al.* 1995, Díaz and Cabido 1997, Lavorel *et al.* 1997). By now, the approaches to classify species range from expert knowledge (Noble and Gitay 1996) to multivariate techniques based solely on trait attributes of species, *e.g.* 'emergent groups'(Kleyer 1999, Gondard *et al.* 2003) with a subsequent testing of the functionality with correlation techniques. While the first approach is not reproducible, the second is criticised for potentially leading to functional groups with low predictive power, because the response of the species to the environment is ignored (Nygaard and Ejrnaes 2004). So far, no classification method has been accepted as a standard procedure (Nygaard and Ejrnaes 2004).

If traits are not only singly analysed but sets of trait states are combined, plant functional groups or syndromes can be created by grouping species with similar response to environmental factors (Lavorel *et al.* 1997). In this study we aimed to describe species compositional changes and identify functional groups in response to land use change in an extensive agro-pastoral system in the region of Alentejo, Southern Portugal. As a consequence of the rapid industrial and socio-demographic changes of the last decades, the characteristic extensive land use practices of these systems are being gradually abandoned and the consequences are still little understood. In this study we therefore focussed our analysis on the functional consequences of abandonment ranging from a current extensive grazing system to scrublands found after 20 years of abandonment.

Traits such as life form, canopy height, dispersal mode and seed mass have been identified as responsive to various types of grassland disturbance, and especially grazing (*e.g.* Fernández

Alés *et al.* 1993, McIntyre *et al.* 1995, Lavorel *et al.* 1999, Peco *et al.* 2005). Since the ecological response of plant communities may be linked to structural traits of species in vegetation that is or has been subjected to these types of disturbances (Lindborg and Eriksson 2005) these traits provide a good starting point to find response traits to abandonment. Plant height and leaf traits associated with resource economy, such as specific leaf area (SLA) and leaf dry matter content (LDMC), have been shown to be relevant to response to grazing abandonment and to succession (Prach *et al.* 1997, Diaz *et al.* 2001, Kahmen and Poschold 2004, Louault *et al.* 2005). Plant height can be related to competition and has generally been reported to increase during succession (*e.g.* Prach *et al.* 1997, Kahmen and Poschold 2004), while small-stature plants seem to be favoured by grazing (Noy-Meir *et al.* 1989, Fernández Alés *et al.* 1993, Lavorel *et al.* 1999, Sternberg *et al.* 2000, Peco *et al.* 2005). The association between small seeded species and disturbance, as well as seed mass increases in response to abandonment have been previously demonstrated for Mediterranean (*e.g.* Fernández Alés *et al.* 1993, Lavorel *et al.* 1999, Garnier *et al.* 2004a) and non-Mediterranean areas (Kahmen and Poschold 2004).

In systems like the one studied, shrubs sprout frequently and are kept at low frequencies by herbivore pressure or clearing (Pereira and Fonseca 2003, Plieninger *et al.* 2004). Therefore, and based on previous studies that have consistently reported the replacement of annuals species by perennials and shrubs during post-disturbance succession in Mediterranean areas (*e.g* Debussche *et al.* 1996, Bonet and Pausas 2004, De Bello *et al.* 2005, Peco *et al.* 2005) we expect life form dominances will strongly shift in response to decreasing land use. The response of other traits is less obvious and only few studies have examined plant trait responses in this or in systems with some similarities (*e.g.* Montado, Dehesa). In a Spanish Dehesa seed mass and dispersal structures were found to be associated with grazing abandonment, where larger seed and the presence of dispersal structures were the traits with the strongest relationship to abandonment (Peco et al. 2005). Abandonment was linked to a

decrease in SLA and an increase in LDMC in South-eastern Mediterranean France (Garnier *et al.* 2004b) as in the Spanish Dehesa (Peco *et al.* 2005). However, these studied communities were mostly composed of herbaceous species and because our sites have a strong woody component the applicability of these findings needs to be validated.

Our analysis of vegetation and functional changes in response to abandonment was organised in three steps. First we analysed vegetation changes using a species-based approach, examining the response of species diversity to abandonment and the positioning of species in the environmental space using redundancy analysis (RDA). Second, we used a functional approach by building functional response groups, thereby identifying the response of groups of species carrying suites of functional traits. Finally we examined changes in community-aggragted, considering traits associated with two ecosystem processes that are key to the provision of services to local stakeholders, flammability and primary productivity (Lavorel and Garnier 2002, Garnier *et al.* 2004b).

METHODS

Study area

The study site (Monte do Vento, Mértola) is located in the Southeast of Portugal, at about 37°48'21.72" N and 7°40'44.96" W.

At the study site, three land use categories were selected to represent a decrease in land use intensity. The first category, 'grazing', is used for extensive grazing by sheep (0.99 CU/ha). The two other categories, 'intermediate succession' and 'advanced succession', represent areas that were abandoned, 10 to 15 years, and 20 or more years ago, respectively. Within each land use category three permanent plots were set. Please refer to Chapter 1 of this dissertation for a more detailed description of the study site and land use categories.

Vegetation and plant traits

Vegetation composition was obtained by recording species presence and percent cover in 15 quadrats randomly distributed within each plot of the three land use categories. Please refer to Chapter 1 for a more detailed description of vegetation sampling.

We selected a set of functional traits that are thought to be relevant to disturbance response and are linked to main plant population processes such as dispersal, establishment and persistence (McIntyre *et al.* 1999). Collecting trait data for all species demands a great effort. Therefore, a compromise must be achieved between effort and accuracy (Pakeman and Quested 2007). Data was collected for the most abundant species (species making at least 80% of plant cover; Garnier *et al.* 2007, Pakeman and Quested 2007), representing 22, 14 and 4 species in 'grazing', 'intermediate' and 'advanced succession' land use categories respectively (see Appendix 1 for a list of measured species). We measured the continuous traits canopy height, specific leaf area (SLA), leaf dry matter content (LDMC) and seed mass for each land use category in which the species occurred, to account for trait intraspecific variability (Garnier *et al.* 2007). The categorical traits life form, pollination mode, type of mycorrhiza, and dispersal mode were collected at the species level independently of land use category since these traits are regarded as stable traits (Cornelissen *et al.* 2003). Measuring traits in different local populations of the same species under different disturbance regimes provides an insight into ecosystem processes that would be hard on a species composition base only (Díaz *et al.* 1999). The measurement of functional traits followed standard methodologies (for a more detailed description see in Garnier *et al.* 2007).

Plant trait measurements

For the measurement of leaf traits, we collected 15 intact, full-grown leaves *per* species. The leaves were collected, as much as possible, from plants in full light situations, or from the outer canopy for tall woody species and stored in moist paper in a cool box until processing.

Water-saturated fresh mass was recorded. Afterwards, leaves were scanned and the projected area determined with ImageJ software (Abramoff *et al.* 2004). Finally, leaves were oven-dried at 60ºC for 72 h and re-weighed. SLA was calculated as the one-sided area of the fresh leaf divided by its oven-dry mass (m^2kg^{-1}). LDMC was calculated as the oven-dry mass of a leaf divided by its water-saturated fresh mass ($mg\,g^{-1}$).

Conopy height was obtained by measuring plant hight at the end of the growing season on 30 individuals *per* species *per* land use.

To determine seed mass, a variable number of seeds, depending on seed size, from 15 individuals of each species was collected. Dispersal structures and fruit flesh were removed as carefully as possible. Seeds were dried to a constant mass at 60ºC before weighing. Small seeds were weighted in groups and the weight of one seed determined by dividing the weight by the number of seeds.

Life form was divided into therophytes, geophytes, hemicryptophytes, chamaephytes and nanophanerophytes (woody species higher than 0.5 m and lower than 2 m). Mycorrhiza type was taken from Gonçalves *et al.* (2005). Dispersal and pollination mode as well as life form were gathered from the literature (Franco 1971; van der Pijl 1982, Franco 1984; Talavera *et al.* 1993; Franco and Afonso 1998; Azcarate*, et al.* 2002, Bastida and Talavera 2002, Koelewijn and Van Damme 2005) and authors' observations.

Community aggregated-traits

Community-aggregated traits (hereafter community-trait) can be used both to detect the average functional response of vegetation environmental factors and to link plant traits to ecosystem functioning (Violle *et.al.* 2007). The values of traits at the community level were calculated from species abundance and plant traits (Garnier *et al.* 2007). For continuous traits this was done as:

$$Trait_{agg} = \sum_{i=1}^{n} p_i \times trait_i$$

where n is the number of most abundant species, p_i is the relative contribution of species i to the community, and $trait_i$ is the trait value of species i. For categorical traits, the relative contribution of each particular attribute was calculated as the sum of relative abundances of species within that attribute. In both cases, this was done for the species that collectively made up at least 80% of plant cover. The two traits considered at the community level were LDMC, in relation to flammability (Saura-Mas and Lloret 2007), and SLA as a marker for specific primary productivity (Garnier *et al.* 2004b).

Statistics

The data consists of lists of species frequencies, species traits and land use categories of the sites.

To evaluate the potential usefulness of different measured traits, we analysed the relationships between pairs of continuous traits on untransformed data, using Spearman rank correlations, calculated for the whole set of measured species. Relationships between continuous and categorical traits were tested by nonparametric analysis (Kruskal-Wallis). Differences in mean species number per quadrat were tested using a randomisation tests (Manly 1997).

To visualise species composition change between land use categories we used an ordination technique. A redundancy analysis (RDA; Canoco 4.5 TerBraak) was performed in which each land use category was represented by a single variable. Contrary to the functional approach, we used all species in the ordination and not only the most abundant. To evaluate the compositional turnover between land use categories we also calculated the Jaccard similarity index (McCune and Grace 2002) between all plots. Subsequently a mean similarity

was calculated for all quadrates from one treatment in combination with all quadrates from the other treatment as well as for subplots within the same treatment.

Differences in community-SLA and community-LDMC between different land use categories were tested using randomisation test (Manly 1997).

Functional group classification

The classification procedure aimed to group species according to their traits in a way that the resulting groups show a significant response to land use. This task involved three parts (Lehsten *et al.* in press). First a large number of classifications of plant groups was generated based on a single trait or a trait combination. Secondly, the response of each plant classification to land use was tested using a null model. In a third step, an optimisation criteria was applied and the optimal classification chosen.

Generating all possible plant group classifications as a first step is not feasible for continuous traits given the enormous amount of possible combinations. To generate a sufficient set of plant classifications it is necessary to limit the maximum number of plant groups per classification. Since there are only four land use classes, we classified the continuous traits into a maximum of three groups (this does not apply for categorical traits for which the full set of possible classification were used). Additionally, the minimum width of each plant group (i.e. difference in the trait values defining the groups) as well as the minimum difference of at least one plant group for different classifications was fixed to account for measurement precision. This value was set to 8 m^2kg^{-1} for SLA, 50 mg g^{-1} for LDMC, 8 cm for canopy height and 0.5 mg for seed mass.

In the second step, the test of response was performed using an extension of the 'fourth corner method' (Legendre *et al.* 1997; Lehsten *et al.* in press) for each classification. Finally, the optimisation criterion of the third step selected the plant classification with the highest number of significant responses. A detailed description of the procedures for generating a

sufficient number of classifications, testing the responsiveness of the classification by a new developed null model and choosing the optimal classification is given in the Appendix. 2

RESULTS

Species response to land use change

The lowest number of species was found for 'advanced succession' (40). In 'grazing' as well as in the 'intermediate succession' a total of 75 species were found. The number of species per quadrat decreased significantly from 'grazing' to 'intermediate succession' (p < 0.001) and from there to 'advanced succession' (p < 0.001, Fig. 1). While the average species number per subplot differed strongly between land use categories, differences in the total richness per land use category were less pronounced.
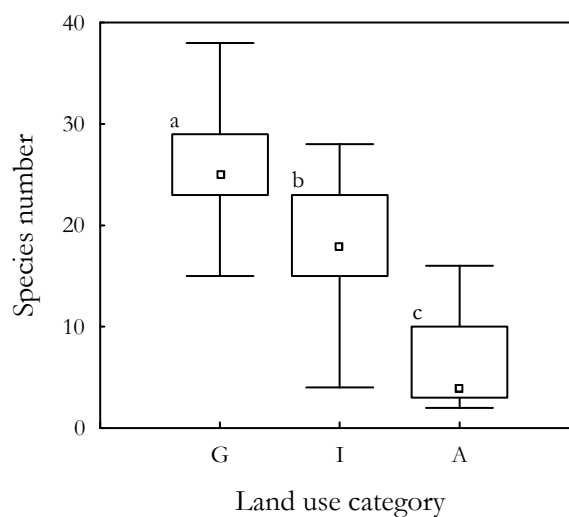
**Figure 1** - Species number per plot. Land use categories. G: grazing, I: intermediate succession, A: advanced succession. The median of species numbers of all categories are significantly different from each other (p<0.001). The sample size is 45 quadrates.

The effect of land use change on the plant community and individual plant species was determined by redundancy analysis. The primary axis of difference between sites was closely

related to 'grazing' (corr. coeff. = 0.95), while the second canonical axis was correlated to 'intermediate succession' (corr. coeff. = 0.93). All canonical axes were significant (Monte Carlo permutation test p<0.001). Figure 2 shows that vegetation composition differs strongly between the land use categories.
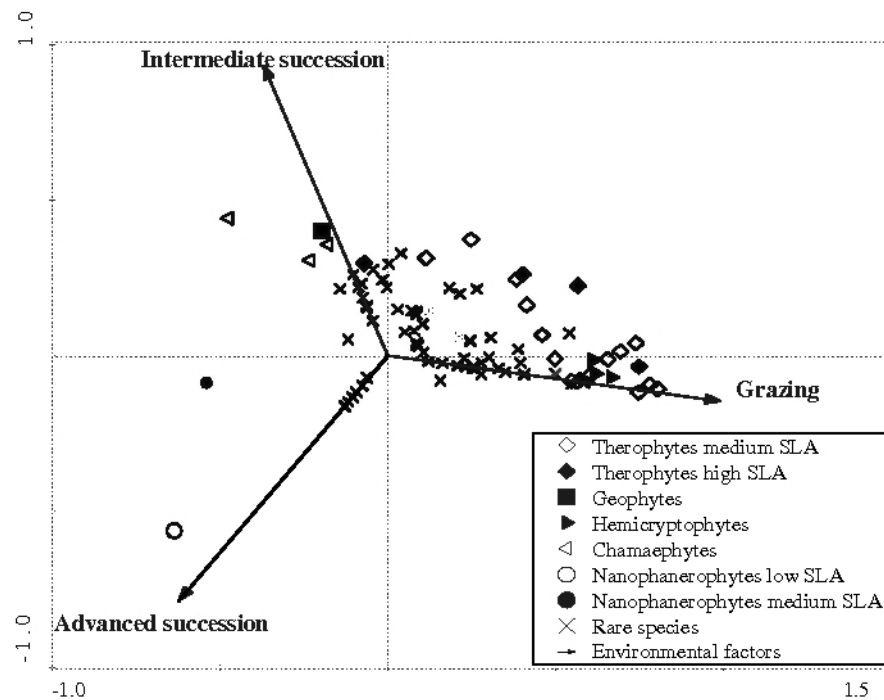
Figure 2 - Species response to land use change. Species are displayed as symbols and factors as arrows. The species symbol is chosen according to the final classification into plant functional response groups. Only the most abundant species (making 80% of plant cover) are classified, remaining species are symbolised by crosses as low cover species. Significance of all factors on plant community is tested by Monte Carlo permutation test (p<0.005).

The strong differences in vegetation composition between land use categories were also shown by the results of the Jaccard similarity index. The average Jaccard similarity for all grazed plots versus all plots with 'intermediate succession' was 0.23, while for 'grazing' versus 'advanced succession' it was 0.06, indicating that on average only 1-2 species were shared by plots between of the two treatments. The results indicated higher compositional turnover

between 'grazing' and 'advanced succession' land use category than between either 'grazing' and 'intermediate succession' or 'intermediate' and 'advanced succession' (average similarity was 0.18). The mean Jaccard index within the treatment was 0.50, 0.31, and 0.42 for 'grazing land', 'intermediate succession' and 'advanced succession' land use categories, repectively.

Associations among traits

A positive correlation was found between LDMC and canopy height ($R^2 = 0.59$, $p < 0.05$) while a negative correlation was found between SLA and canopy height ($R^2 = 0.41$, $p < 0.05$). No significant correlations were found between other combinations of continuous traits. Life form was associated with canopy height ($p < 0.05$), SLA ($p < 0.05$) and LDMC ($p < 0.05$), but not with the other traits. This association is mostly due to growth form. Herbaceous species (therophytes, geophytes and hemicriptophytes) tend to be smaller ($p < 0.001$), have lower LDMC ($p<0.01$) and higher SLA ($p<0.01$) than shrubs (chamaephytes and nanophanerophytes). No associations between other combinations of traits were found.

Response of single plant traits

In the first step we optimised functional response groups for each trait singly and calculated the response to the land use category (see Table 1). A valid functional classification was found for each of the investigated traits. However, for some traits the classification contained many groups with a non significant response to some of the land use categories.

The response of canopy height was related to land use intensity. Small plants (canopy height < 18 cm) had a positive response to grazing while medium sized plants with a canopy height between 18 cm and 71 cm responded positively to intermediate and advanced succession. Plants taller than 71 cm responded positively only to advanced succession.

Table 1 - Optimal classification and response of species according to single traits. A plus sign (+/++) denotes a positive response at the p <0.05 / p < 0.01 level. Minus signs indicate negative a response, n.s. is used if the p-values were above 0.05. A positive response indicates that the plant groups reach a higher coverage than expected by the null model. Land use categories: G: grazing; I: intermediate succession; A: advanced succession; life forms: thero: therophytes; geo: geophytes; hemi: hemicryptophytes, shrub: chamaephytes and nanophanerophytes; mycorrhiza: AM: vesicular arbuscular mycorrhiza; ECM: ectomycorrhiza; dispersal mode: U- unassisted, W-wind, E-exo-zoochory and pollination: S-self-pollinated, W- wind, I- insect, M- mixed.

Trait	Attribute	G.	I.	A.
LDMC	<270	++	- -	- -
(mg g⁻¹)	270-470	- -	++	- -
	>470	- -	-	++
Life form	The	++	- -	- -
	Geo	- -	++	- -
	hCr	++	- -	- -
	Ch	- -	++	- -
	nPh	- -	n.s.	++
Seed mass	<0.2.1	++	- -	- -
(mg)	>2.1	- -	++	++
SLA	<12	- -	- -	++
(m² kg⁻¹)	12-16	- -	++	- -
	>16	++	- -	- -
Pollination	S	++	n.s.	- -
	W	++	- -	- -
	I	- -	n.s.	++
	M	++	n.s.	- -
Mycorrhiza	no	++	- -	- -
	AM	++	++	- -
	ECM	- -	- -	++
Height	<18	++	- -	- -
(cm)	18-71	- -	++	++
	>71	- -	- -	++
Dispersal	U	- -	++	++
	W	++	- -	- -
	E	++	n.s.	- -

Seed mass was positively related to abandonment with low seed mass being found in 'grazing' and higher seed mass in the other two land use categories. Specific leaf area showed a negative response to decreased levels of land use and LDMC was positively related to abandonment.

Therophytes showed a positive response to 'grazing', together with hemicryptophytes. The only geophyte, *Carlina corymbosa,* and chamaephytes responded positively to 'intermediate succession' while nanophanerophytes had a positive response to 'advanced succession'.

The pollination and dispersal mode showed only a weak response to the land use category. All species except *Rumex acetosella, Silene gallica* and *Cistus ladanifer* had arbuscular mycorrhiza. Hence, arbuscular mycorrhiza showed a positive response to all land use categories, except 'advanced succession', which is dominated by *Cistus ladanifer*, the only species with ecto-mycorrhiza. The species without mycorhiza (*Rumex acetosella, Silene gallica*) responded positively to grazing.

Plant Functional Response Groups

The previous set of analyses showed that several single traits responded strongly to land use. Considering the low number of land use categories we decided to combine not more than two traits to derive final functional response groups (FRGs). We tested all combinations of the categorical trait life form with each of the four continuous traits. The combination of life form and SLA resulted in the classification with the highest explanatory value according to our optimisation criterion (Table 2). All groups responded significantly to all land use categories. This classification also had the lowest number of positive responses to different treatments and hence the clearest plant type - treatment allocation.

Table 2 - Optimal classification of functional response groups using two traits. A plus sign (+/++) denotes a positive response at the p < 0.05 / 0.01 level. Minus signs indicate a negative response. Land use categories: G: grazing; I: intermediate succession; A: advanced succession; Life forms: Th: therophytes; Geo: geophytes; hCr: hemicryptophytes, Ch: chamaephytes, nPh nanophanerophytes

Trait attributes of FRG		Land use category			Species per group	Height (cm)	Seed mass (g)
Life form	SLA (m² kg⁻¹)	G.	I.	A.		± sd	±sd
Th	12-30	++	- -	- -	16	10.8±6.7	0.63±0.90
Th	>30	++	+	- -	5	11.6±6.5	0.77±1.0
Geo	12-30	- -	++	- -	1	21.4	1.24
hCr	12-30	++	- -	- -	3	9.6±5.8	0.22±0.23
Ch	12-30	- -	++	- -	3	61.4±14.9	0.76±0.77
nPh	<12	- -	- -	++	1	136	0.2
nPh	12-30	- -	+	++	1	77	2.5

Therophytes with medium SLA were related to 'grazing', while therophytes with high SLA occurred both in 'grazing' and 'intermediate succession' land use categories. All geophytes, hemicryptophytes and chamaephytes have a medium SLA. Their response was therefore similar to the response derived in the single trait analysis of life form. Nanophanerophytes were separated into medium SLA (*Genista hirsuta*), which was related to intermediate and advanced succession, and low SLA (*Cistus ladanifer*), which showed a positive response to 'advanced succession' only. Since the classification was made using treatment specific trait measurement (Garnier *et al.* 2007), a single species could belong to more than one functional response group. For example *Brachypodium distachyon* and *Tolpis barbata* were classified as belonging to two groups therophytes with medium SLA, in 'grazing', as well as therophytes with high SLA, in 'intermediate succession', since the measured SLA values varied between the treatments. In Fig. 2, they are assigned to the Therophytes with high SLA.

Response of Community-aggregated traits

At the community level, LDMC was lowest in 'grazing' and significantly increased (p<0.001) thereafter with decreasing human impact (see Fig. 3). On the contrary, SLA significantly decreased (p<0.001) with abandonment as herbaceous dominated communities are progressively replaced by shrub dominated communities. This replacement of species with high SLA and low LDMC with species of opposite traits suggests an increase in flammability and a decrease in productivity and decomposition.

DISCUSSION

Species response to land use change

Land use change in the in the study site, as in other Mediterranean ecosystems (Debussche *et al.* 1996, Peco *et al.* 2005) is mostly associated with succession after abandonment and thus with secondary successional vegetation change. As indicated by the mean Jaccard index, the compositional turnover is always greater between than within land use types. Abandonment had therefore a strong effect on vegetation composition in Montado areas, with the strongest effects being observed during the initial stages after the abandonment of grazing. Relatively large changes in species composition in relation to abandonment have also been reported in other studies in Mediterranean region (Noy-Meir *et al.* 1989, Peco *et al.* 2005).

Similarly to species composition, species richness was also strongly affected by abandonment. Not surprisingly the highest species diversity was associated to grazing. Grazing, even if light and intermittent, considerably increases the number, size and diversity of gaps in the grassland allowing the germination and establishment of species that were not able to do so in the closed canopy (Noy-Meir *et al.* 1989). The decrease in species diversity with

decreasing land use intensity is mostly a consequence of the replacement of short lived herbaceous species by shrubs (see Debussche *et al.* 1996).
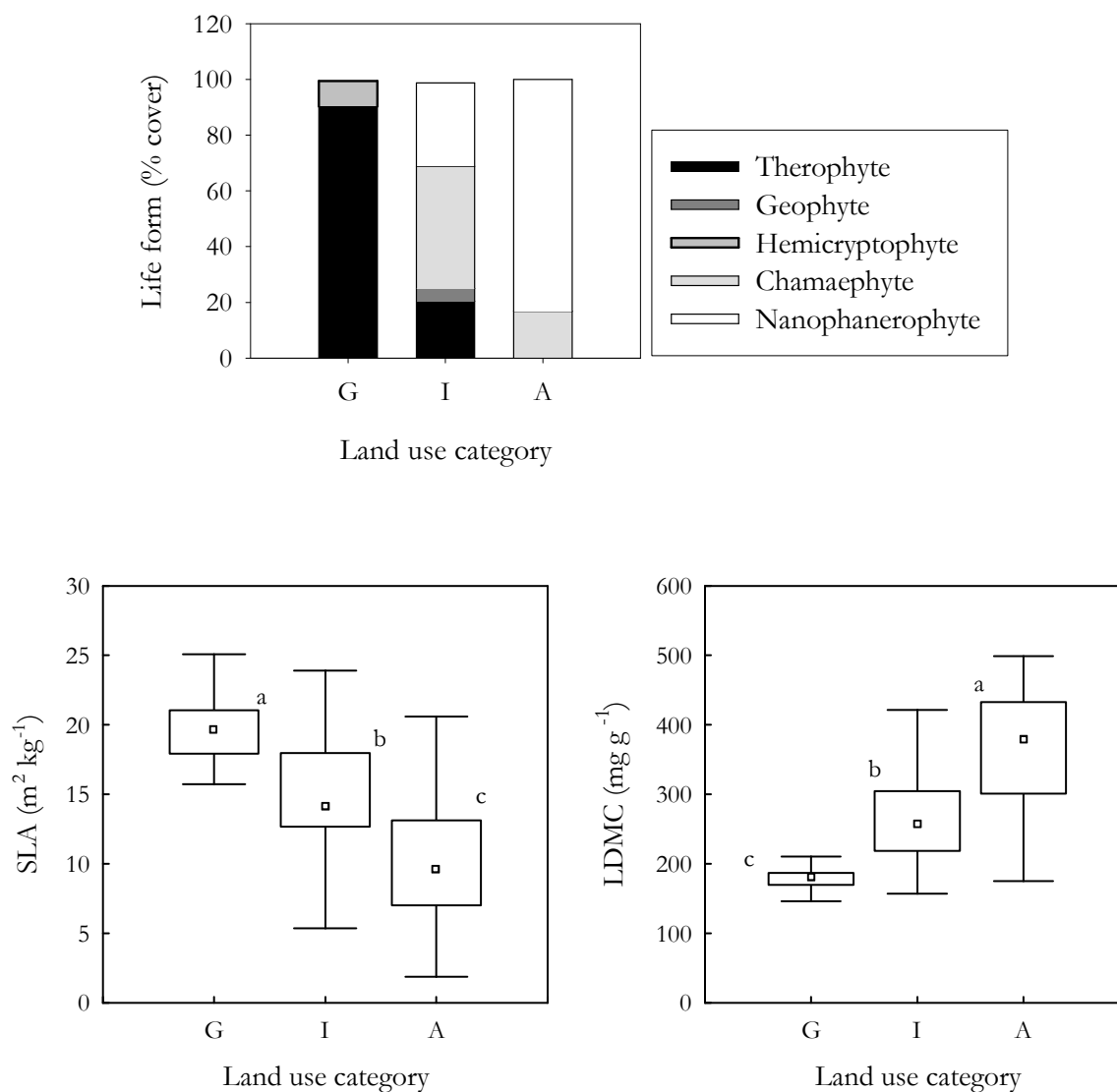

Figure 3 - Community-aggregated traits, trait values are weighted by percentage cover. Significant differences (p<0.001) in median values are signalled by different letters. Land use categories: G – grazing, I – intermediate succession, A – advanced succession. Only the most abundant species (making 80% of total cover) are used.

Response of individual plant traits

Our results confirm that small stature and short life span are traits linked to the ability to cope with disturbance, while taller plants and the chamaephyte and nanophanaerophyte life forms are related to abandonment in the Mediterranean (*e.g.* De Bello *et al.* 2005, Peco *et al.* 2005). Small plants were more frequent in grazed plots than in abandoned plots, even when only herbaceous species were taken into account (data not shown). The decrease in frequency and intensity of disturbance results in the accumulation of standing biomass and in the increase in the competition for light (Grime 2001) favouring plants with a taller canopy. Contrary to other studies, hemicryptophytes had a small representation in the vegetation and were not favoured by abandonment. Our findings seem to support the hypothesis that in extensive agro-pastoral systems lack of recolonization by hemicryptophytes results from the absence of seed source in the landscape (Lavorel *et al.* 1999). Environmental filters act by removing species that lack traits attributes for persisting under a particular set of conditions (Diaz *et al.* 1998). Centuries of intensely managed agriculture may have eliminated disturbance-intolerant species and may have acted as such a filter.

With regards to regenerative traits, our study shows that grazing favours species with small seeds while abandonment favours species with heavier seeds. Small seed size has been related to larger seed production (Turnbull *et al.* 1999), better chances of surviving herbivore gut passage (Pakeman *et al.* 2002, Peco *et al.* 2005) and to seed persistence in the soil (Thompson *et al.* 1993, Peco *et al.* 2003), an essential characteristic in grazed Mediterranean annual pastures where bare ground is open to recruitment every autumn and rainfall is variable (Ortega *et al.* 1997). The increase in seed mass with abandonment time has also been reported previously (*e.g.* Lavorel *et al.* 1999, Garnier *et al.* 2004a, Peco *et al.* 2005). This response may be related to the fact that plants with larger seeds generally germinate better, are better able to establish and survive under a wide range of conditions (Westoby 1998, Turnbull *et al.* 1999).

Contrary to findings by Peco *et al.* (2005) in Dehesa grasslands, unassisted seed dispersal was more related to abandoned areas and the presence of dispersal structures was associated to disturbance. Dispersal by wind is frequent among species that are relatively tall within their respective habitat. For understorey plants wind dispersal is less advantageous than other modes (Wilson and Traveset 2000). At our study sites wind dispersal becomes less important as abandoned areas progress toward a taller and denser canopy. Similarly, animal dispersed species, mostly dispersed by adhesive structures or accidental ingestion of seed by grazers, decreased in importance with increasing time of abandonment.

Finally our analysis of leaf traits confirmed that species with high SLA and low LDMC are more frequent in grazed areas, and that SLA decreases while LDMC increases with abandonment. Likewise, Garnier et al. (2004b) observed a replacement of fast-growing species (high SLA and low LDMC) which dominate the early stages following abandonment, by slower growing species (low SLA and high LDMC) as succession proceeds in abandoned vineyards. This consistent response is of particular interest as it confirms for a site with a strong woody component results previously obtained for herbaceous vegetation.

Plant functional response groups

In order to identify plant functional types in relation to secondary succession in the study site we tested all combinations of life form, seed mass, canopy height, SLA, type of pollination, mycorrhiza and dispersal. The combination of the two traits, life form and SLA was found to be optimal. Therophytes with high SLA were associated to both grazing and intermediate succession, while therophytes with medium SLA were associated to grazing. Therophytes with medium SLA are the most abundant group in grazing land use category (74.8 % of plant cover) and were the group most strongly affected by abandonment. Lower SLA is usually associated with low palatability and therefore to mechanisms of grazing

resistance (Cornelissen *et al.* 1999). Although the response of plant species to grazing may be diverse, in situations of water and nutrient scarcity such as those of the present study grazing avoidance should be favoured against grazing tolerance (Diaz *et al.* 2001). Plants found under grazing generally have prostate growth, which supports the hypothesis of grazing avoidance.

Therophytes with high SLA appeared less sensitive to abandonment than therophytes with medium SLA. Abandonment from grazing at our sites resulted in an increase in vegetation height and biomass, which holds true even when shrubs are excluded from the analyses (unpublished data). Hence, less light is available for therophyte plants growing in intermediate succession when compared to grazing. Specific leaf area is known to be strongly affected by light and to increase at lower light levels (Poorter *et al.* 2006). The observed decrease in community weighted mean SLA in intermediate succession as compared to grazing may be attributed to a combination of species turnover, as indicated by the results of the Jaccard index, and of within species variability in trait values (De Bello *et al.* 2005). The land use category intermediate succession is indeed dominated by chamaephytes with medium SLA (e.g. *Lavandula stoechas*, *helychrisum stoechas*, *L. viridis*), but all other groups, except hemicryptophytes with medium SLA, also occur to a smaller extent. Measuring traits which were not considered as stable i.e. canopy height, SLA, LDMC and seed mass for each land use category at which the species occurred (Garnier *et al.* 2007) made it possible to detect intra-specific as well as inter-specific responses. For example, *Brachypodium distachyon* and *Tolpis barbata* were classified as belonging to therophytes with medium SLA in grazing land use category and as therophytes with high SLA in intermediate land use category. Our methodology for analysis, which focuses on functional groups with different combinations of trait values as opposed to mean responses of single traits, was able to detect such responses although their direction was opposite to the community mean (Pillar *et al.* 1999, Louault *et al.* 2005). We thereby also highlighted changes in functional diversity in response to post-grazing

succession (de Bello *et al.* 2006). Likewise, our analyses separated nanophanerophytes into medium SLA (*Genista hirsuta*) and low SLA (*Cistus ladanifer*), and both groups were related to advanced succession land use category showing the coexistence of species with different strategies in the same community. *Genista hirsuta* is a summer deciduous species while *Cistus ladanifer* is semi-deciduous. Deciduous leaves are characterised by higher SLA compared to semi-deciduous (Villar *et al.* 2006).

Response of community-aggregated traits

Traits of dominant plants are known to strongly influence the functioning of ecosystems (Grime 1998). Important traits affecting ecosystem processes are those that relate to availability, capture and use of soil resources that affect the trophic structure and influence the frequency, severity and extent of fire (Chapin III and Walker 1997). Effects of traits of dominant species have recently been demonstrated for key ecosystem processes such as decomposition (Aerts and Chapin 2000, Garnier *et al.* 2004b, Kazakou *et al.* 2006) and productivity (Aerts and Chapin 2000, Garnier *et al.* 2004a). High community-SLA and low community-LDMC tend to be associated to higher productivity and faster decomposition. The observed decrease in community-SLA and increase in community-LDMC after abandonment therefore suggest a decrease in productivity and decomposition rate with abandonment of grazing, with likely consequences on the carbon and nutrient cycles. LDMC is also an indicator of flammability (Lavorel and Garnier 2002, Garnier *et al.* 2004a, Saura-Mas and Lloret 2007). The replacement of herbaceous species with taller, more flammable shrub species, having higher biomass (more fuel), can be expected to lead to an increase in frequency as well as severity of wild fires. Additionally, shrub encroachment can lead to a further deterioration of pasture quality and consequently to more abandonment. On the other hand scrubland areas provide habitat for many bird and mammal species, some of which are of

interest for hunting. In fact, increase of scrubland will increase local diversity of breeding birds, even though it decreases the conservation value of the area as farmland species of high conservation value are replaced by widespread scrubland species (Moreira *et al.* 2005). Abandonment therefore leads to a complex set of changes in ecosystem processes that local people derive services from, with concurrent positive and negative impacts that will need to be taken into account for future management.

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CHAPTER 3

ABOVEGROUND BIOMASS AND PRODUCTIVITY IN AN EXTENSIVE AGRO-PASTORAL
SYSTEM: FROM HERBACEOUS TO SHRUB DOMINATED COMMUNITIES

INTRODUCTION

The agro-pastoral systems of South Portugal, as most Mediterranean rangelands, have originated from natural forests following the removal of trees by human activities such as clearing, burning and grazing (Pulido *et al.* 2001). When agricultural practices decrease or stop, succession generally leads to the invasion by woody plants. In the studied system abandonment lead to a change in vegetation structure and composition that resulted in the replacement of herbaceous dominated communities with shrub dominated communities. Shrub encroachment has been reported in many grassland and savanna ecosystems in North and South America, in Africa and Australia (*e.g.* Van Auken 2000, Bowman 2002, Hughes *et al.* 2006), often as a result of overgrazing of herbaceous species or changes in fire regime (Van Auken 2000, Asner *et al.* 2004). It has also been reported in Greek rangelands as a consequence of decreasing land use intensity (Karakosta and Papanastasis 2007, Zarovalli *et al.* 2007). Woody encroachment is likely to cause substantial alterations in the sequestration and cycling of carbon and nitrogen (Briggs *et al.* 2005, Hughes *et al.* 2006) because grasslands are generally expected to have high biomass turnover, productivity and nutrient cycle, and only moderate capacity for carbon sequestration in biomass when compared to woody communities (Díaz and Cabido 1997, Gill and Burke 1999). Increases in above-ground carbon storage with shifts to woody vegetation have been reported by some authors (*e.g.* Jackson *et al.* 2002, Asner *et al.* 2003, Hughes *et al.* 2006). Several specific aspects of ecosystem function, among which is above-ground net primary productivity (ANPP) were directly affected by the relative abundance of the grass and shrub functional types in a study in the Patagonian steppe (Aguiar *et al.* 1996). Above-ground primary productivity decreased as shrubs increased because shrubs did not fully compensate for the decrease in grass production (Aguiar *et al.* 1996). Zarovalli *et al.* (2007), and Karakosta and Papanastasis (2007) noted a decrease in herbaceous biomass and production as woody species cover increased. Considering the productivity of

herbaceous and woody components together, Huenneke *et al.* (2002) reported a small but significant decrease in overall productivity of desertified shrub systems of New Mexico in comparison to grasslands. However, there is still a degree of uncertainty regarding how biomass and productivity of herbaceous and woody components interact in response to shifts in plant life form composition (Hughes *et al.* 2006), particularly in Mediterranean, where such studies are scarce. Studies in Mediterranean areas (Karakosta and Papanastasis 2007, Zarovalli *et al.* 2007) suggest a possible decrease in herbaceous ANPP but this may be compensated by shrub productivity, resulting in no change in ANPP, or more than compensated, resulting in an increase in ANPP (Huenneke *et al.* 2002, Reich *et al.* 2001, House *et al.* 2003)

Our question is focused on the effect of abandonment and, more specifically, the shift from herbaceous to shrub dominated communities, on the community above-ground biomass and ANPP. We hypothesized that the change in life form dominance would affect both biomass and productivity but while the total biomass was expected to increase, because shrubs have larger biomass than herbaceous species, the effects on ANPP were less clear.

METHODS

Study site

The study site (Monte do Vento, Mértola) is located in the Southeast of Portugal, at about 37°48'21.72" N and 7°40'44.96" W.

At the study site, three land use categories were selected to represent a decrease in land use intensity. The first category, 'grazing', is used for extensive grazing by sheep (0.99 CU/ha). The two other categories, 'intermediate succession' and 'advanced succession', represent areas that were abandoned, 10 to 15 years, and 20 or more years ago, respectively. Within each land

use category three permanent plots were set. Please refer to chapter 1 of this dissertation for a more detailed description of the study site and land use categories.

Sampling

Herbaceous Aboveground Biomass and ANPP

The methods chosen to measure above-ground biomass and ANPP in each land use category were different for shrub and herbaceous vegetation.

For herbaceous vegetation, the sampling of plant above-ground biomass and ANPP followed "Method 1" of Scurlock *et al.* (1999). This method estimates above-ground biomass and ANPP based on a single harvest at the peak of live biomass. It assumes that any standing dead matter or litter was carried over from previous years, and death in current year is negligible. This method was considered adequate for the studied communities, which are mostly composed of annual species.

One harvest of above-ground biomass was conducted in April-May 2004 to assess maximum standing biomass (Scurlock *et al.* 2002). Eight quadrates of 0.25 x 0.50 m were sampled in each of the three permanent plots in 'grazing' and 'intermediate succession' land use categories, resulting in an area of approx. 1 m^2 sampled per plot. Each plot was considered a replicate, resulting in three replicates per land use category (Garnier *et al.* 2007). For each quadrate, all above-ground material (live and dead) was collected by clipping at ground level. Live material was separated from the dead and the two fractions were oven-dried to constant mass at 60ºC and weighed (Garnier *et al.* 2007). Live material was used to determine above-ground live biomass and ANPP.

Shrubs Aboveground Biomass and ANPP

Shrub measurement techniques (*e.g.* Vora 1988, Fernández *et al.* 1991, Armand *et al.* 1993, Jobbágy and Sala 2000, Sternberg and Shoshany 2001, Navarro and Oyonarte 2006) used in other semi-arid areas of the world were examined with the objective of adapting a suitable, non-destructive, and relatively simple and accurate method to the shrub species in the study area. Although a large number of variables could be used to predict biomass and ANPP, variables that express the size of the crown appeared to be the most useful (Murray and Jacobson 1982).

The collection of data for the estimation of shrub above-ground biomass and ANPP consisted of two steps.

First, in order to establish regression equations for the estimation of above-ground biomass and ANPP, we measured and harvested 6 to 12 individuals of the shrub species *Cistus ladanifer*, *Genista hirsuta*, *Helichrysum stoechas*, *Lavandula stoechas* and *Lavandula viridis*, which were the dominant shrub species in the studied plots (other shrub species present had negligible cover. See Appendix 1). For each individual we measured: (1) Total height, defined as the maximum vertical distance from the ground level to the highest point of the plant; (2) Crown diameter, as the mean of two perpendicular diameters. From these measurements we determined crown area and volume. Crown volume was determined, for each species, using the formula of the solid that appeared to give the best fit of the natural shape of the crown. The geometric shape that best fitted the sampled species was the inverted cone. Assuming a cone shape, plant volume (V) was calculated as $V = \pi/3 r^2 h$, where *r* is the crown radius and *h* is total height.

In the laboratory, the harvested individuals were separated into green leaves and current year shoots, woody parts, and dead material. The different fractions were oven-dried to constant mass at 60°C and weighed. Green leaves and current year shots were considered as

current year production (Fernández *et al.* 1991, Alldredge *et al.* 2001) and used as an estimate of ANPP. The measured variables were regressed against dry weight (see description below).

Second, three quadrates of 6 m^2 per plot were sampled in 'intermediate' and 'advanced succession' land use categories. In each of these quadrates, we counted and measured (as above) all the individuals of each shrub species present. Plant biomass within these quadrates was estimated by applying the developed regression equations (see description below). Total above-ground biomass values for each species in a quadrate were summed to obtain biomass per square meter. The three quadrates in each plot were averaged to yield a single value per plot.

Above-ground net primary productivity was calculated as a proportion of the total plant biomass based on the mean values obtained from the individuals collected (0.25% in *Cistus ladanifer*, 0.14% in *Genista hirsuta*, 0.58% in *Helichrysum stoechas*, 0.40 % in *Lavandula stoechas* and 0.27% in *Lavandula viridis*).

For 'intermediate succession' plots the above-ground biomass and ANPP of the herbaceous layer was measured as for the 'grazing' plots while for the shrub layer we proceeded as for 'advanced succession' plots. The total above-ground biomass and ANPP per plot was obtained as the sum of the herbaceous and shrub layers.

Statistics

Regression equations

Curve estimation regression models were used to determine the type of regression that best described the relationship, in each species, between measured variables (height, volume and area) and dry weight. The variable that best predicted dry weight was volume (V) in *Cistus ladanifer*, *Genista hirsuta*, and *Helichrysum stoechas*, and area (A) in *Lavandula stoechas* and *Lavandula viridis*. The curves that best described these relationships were linear and power. The best

equation for each species was selected based on the coefficient of determination. The equations selected, linear for *Cistus ladanifer* and *Helichrysum stoechas,* and power for *Genista hirsuta*, *Lavandula stoechas* and *Lavandula viridis* are presented in Table 1. The coefficients of determination for the selected equations ranged from 0.889 to 0.987 (Table 1).

Table 1 - Regression equations (Wt = estimated weight in g, V = Canopy volume in cm^3, A = canopy area in cm^2), coefficients of determination (r^2), significance (p), and number of samples (n).

	Equations	r^2	p	n
Cistus ladanifer	$W_t = 90.819 + 0.002*V$	0.953	<0.001	12
Genista hirsuta	$W_t = 0.003*V^{1.027}$	0.973	<0.001	6
Helichrysum stoechas	$W_t = 7.009 + 0.003*V$	0.987	<0.001	6
Lavandula stoechas	$W_t = 0.213*A^{0.872}$	0.960	<0.001	6
Lavandula viridis	$W_t = 0.009*A^{1.366}$	0.889	<0.001	6

Data analysis

Differences in mean above-ground live biomass, ANPP and total above-ground dead biomass between land use categories were tested, on untransformed data, with the ANOVA or students t-test, according on the number of land use categories that were under analysis. The Pearson's correlation coefficient was used to test for correlations between above-ground live biomass and shrub cover, and ANPP and shrub cover. All statistical analyses were performed with SPSS 14.0 for windows (SPSS, Inc. Chicago, IL).

Methodological considerations

In this study, annual herbaceous ANPP was estimated by the peak of green biomass. Studies in other system indicate that if the purpose is to estimate annual primary production, peak of green biomass is one of the methodological options with the smallest error (Jobbágy

and Sala 2000). Furthermore, annual herbaceous communities fulfil the assumption that live biomass that was not carried over from previous years (Scurlock *et al.* 2002). In the case of shrubs, we assumed that current year leaf and twig biomass accounted for annual ANPP. A slight underestimation of shrub ANPP may be expected since we did not account for stem diameter changes in older branches. The use of current year biomass accumulation at the end of the growing season as an estimate of ANPP has been successful in other studies were shrub ANPP was estimated (Jobbágy and Sala 2000). Additionally, we calculated ANPP as a proportion o total shrub biomass. We compared the obtained from the collected individuals with those obtained in other studies when these were available. Values found in other studies ranged from 0.21 to 0.27 in *Cistus ladanifer* (Simões 2002, Navarro and Oyonarte 2006), and from 0.21 to 0.35 in *Lavandula stoechas* (Armand *et al.* 1993, Navarro and Oyonarte 2006), were about 0.15 in *Gennista* spp (Navarro and Oyonarte 2006), and 0.46 in *Helichrysum italicum*. Values found in the present study were generally within the range of those reported for the same species or genus, except in the case of *Lavandula stoechas* and *Helichrysum stoechas* which showed higher values. Even though one should be aware of the possible errors resulting from the methodology used, we consider that it was adequate for the purpose of this study.

RESULTS

Aboveground biomass and ANPP

Above-ground biomass differed significantly between all land use categories, from a minimum of 194.71 gm^{-2} in 'grazing' to a maximum of 1164.89 gm^{-2} in 'advanced succession' (Fig. 1).

The increase in above-ground biomass after abandonment was strongly related to the increase in shrub cover (Table 3), particularly *Cistus ladanifer*.
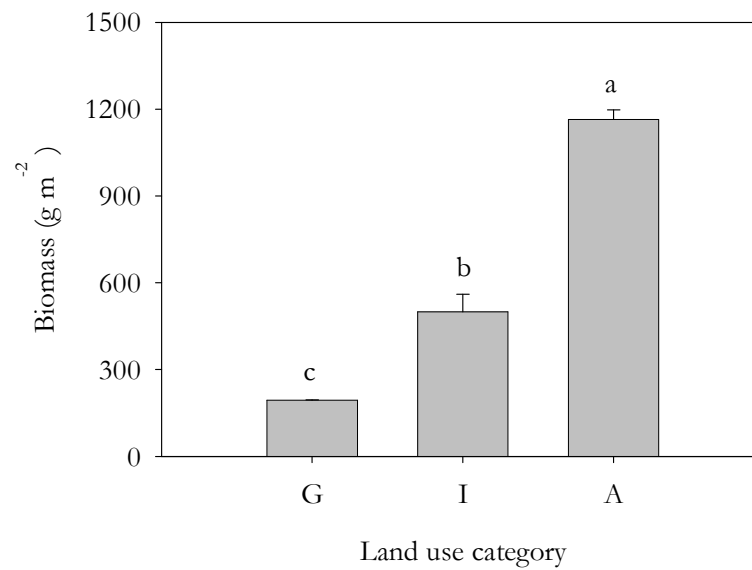
Figure 1 - Above-ground biomass in grazing (G) and intermediate (I) and advanced (A) succession land use categories (F = 115.07, p < 0.0001, n=9). Values in intermediate succession represent the sum of plant biomass in the herbaceous and shrubs layers.

Table 3 - Relationships (Pearson's correlation coefficients) between above-ground biomass, ANPP and shrub cover. Significance levels: *p < 0.05, **p < 0.01

	Shrub cover	Cover of *C. ladanifer*
Above-ground biomass	0.887**	0.985**
$ANPP_{Total}$	0.70*	0.885**
$ANPP_{shrubs}$	0.858*	0.972**

Aboveground net primary productivity ranged from 194.71 gm^{-2} in 'grazing' to 271.15 gm^{-2} in 'advanced succession' (Fig. 2). When the herbaceous and shrub layers were separately analysed (Fig. 3 and 4), intermediate succession had a significantly lower herbaceous ANPP when compared to 'grazing' (Fig. 3; t = 4.14, p < 0.05), and significantly lower shrub ANPP when compared to 'advanced succession' (Fig. 4; t = 5.38, p < 0.05).

Additionally, data for the total above-ground dead biomass of the herbaceous layer were analysed. This analysis showed a significantly higher (Fig. 3; t = 3.58, p < 0.05) amount of dead material in 'intermediate succession' when compared to grazing. The increase in ANPP of the shrub layer was strongly related with the increase in the cover of *Cistus ladanifer* (Table 3). As shown in Fig. 4, shrub ANPP and *Cistus* ANPP significantly increased from 'intermediate' to 'advanced' succession while the sum of ANPP of the other shrubs did not change significantly.
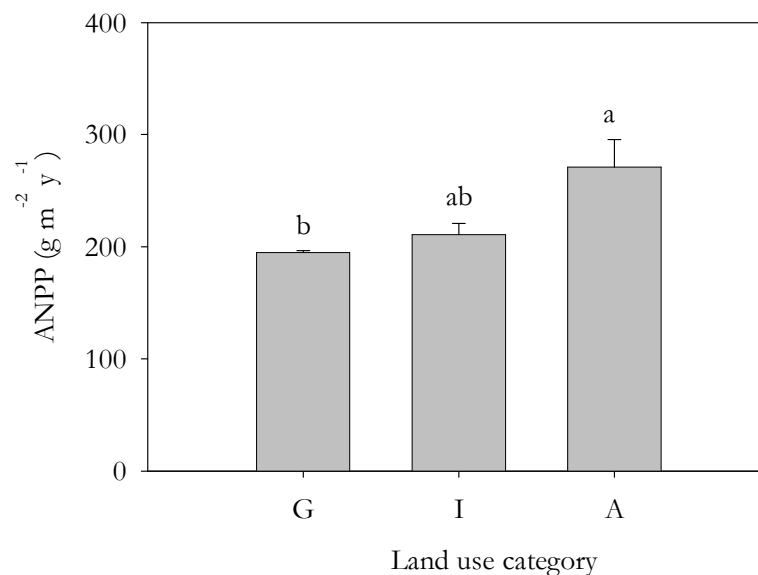

Figure 2 - Above-ground net primary productivity (ANPP) in grazing (G), intermediate (I), and advanced (A) succession land use categories (F = 6.95, p < 0.05, n=9). Values in intermediate succession represent the sum of plant biomass in the herbaceous and shrub layers.
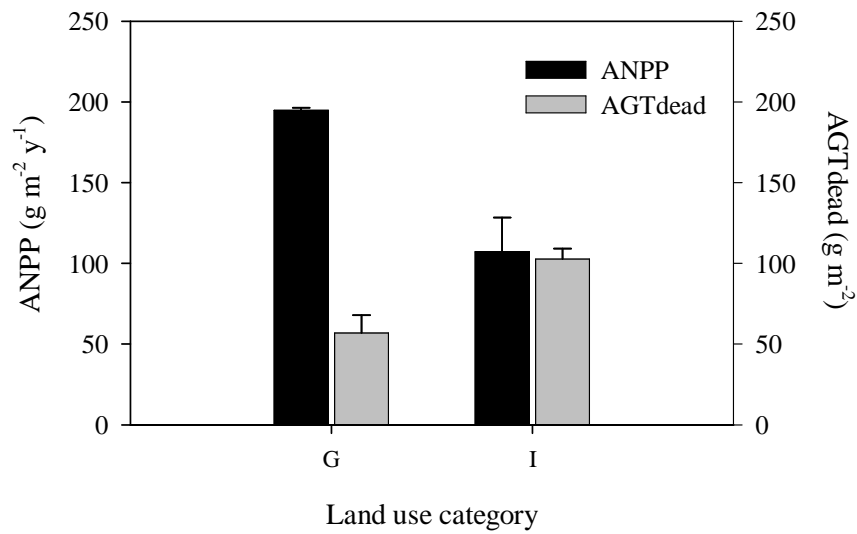
Figure 3 - Above-ground net primary productivity (ANPP) and above-ground dead plant material (AGTdead) of the herbaceous layer in grazing (G) and intermediate succession (I) land use categories. Both variables are significantly different among areas (ANPP: t = 4.14, p < 0.05, n=6; AGTdead: t=3.58, p < 0.05, n=6).
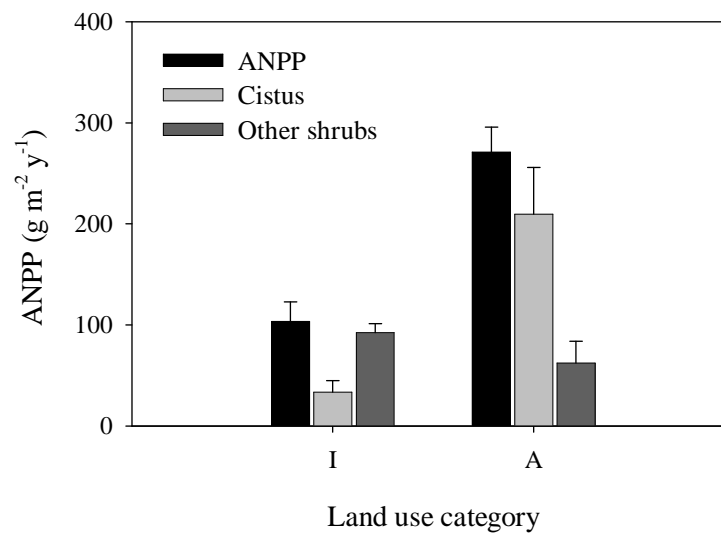

Figure 4 - Above-ground net primary productivity of all shrubs sampled (ANPP), of *Cistus ladanifer* (Cistus) alone, and the sum of other shrub species (Other shrubs) in intermediate (I) and advanced succession (A) land use categories (ANPP: t = 5.38, p < 0.01, n=6; Cistus: t =3.41, p < 0.05, n=6; Other shrubs: t =1.29, p > 0.05, n=6).

DISCUSSION

Above-ground biomass of shrub communities showed lower values than those reported for Mediterranean shrub communities (2190 to 4600 g m^{-2}, Ehleringer and Mooney 1983) and than those found by Simões (2002) for the above-ground biomass of *Cistus ladanofer* in a study in South Portugal (1605 to 1936 g m^{-2}). Above-ground net primary productivity of shrub communities showed higher values than those reported for Mediterranean shrub communities (110 to 130gm^{-2}, Ehleringer and Mooney 1983) but lower than reported by Simões (2002) for the sum of green leaves and current year shoots of *Cistus ladanifer* in a study in South Portugal (420.6 to 538.8 g m^{-2}).

The herbaceous species that generally dominate early secondary successional sites return most of their biomass to the soil each year. As perennial species, particularly shrubs, increase in abundance, biomass increases more rapidly because woody species retain a larger proportion of their biomass than do herbaceous species (Chapin III *et al.* 2002). Our results showed an increase in total above-ground biomass with abandonment time and are therefore consistent with what would be expected following the replacement of herbaceous with woody species. The increase in above-ground biomass through succession in our study was strongly related to the increase in shrub cover. Differences in biomass thus reflect the changes in the dominant life form.

When only herbaceous species were taken into account, the above-ground biomass and ANPP decreased from 'grazing' to 'intermediate succession' in our study. Herbaceous biomass and production were also significantly reduced as shrub cover increased in Greek rangelands (Karakosta and Papanastasis 2007, Zarovalli *et al.* 2007). The accumulation of dead material has important implications for productivity and community composition by intercepting light, and shading seeds and seedlings (Facelli and Pickett 1991, Grime 2001). In herbaceous communities, most of the aerial biomass is allocated to short lived organs resulting in a rapid

accumulation of litter in early stages of succession (Facelli and Pickett 1991). The higher mass of herbaceous above-ground dead material in 'intermediate succession' is likely to be a consequence of the absence of grazing, which together with the increase in shrub cover may explain the decrease in both biomass and ANPP of herbaceous vegetation in this area. A negative effect of larger amount of above-ground dead material on ANPP in ungrazed plots was also reported by Altesor *et al.* (2005) for temperate sub-humid grasslands.

Different types of vegetation can attain similar aboveground productivities because of compensating factors in the productive structure of the community. In a study which encompassed forest, scrub, and herbaceous Mediterranean communities, Ehleringer and Mooney (1983) reported a difference between these communities of 30-fold in aboveground biomass and only a 2-fold difference in aboveground productivity. In a temperate oak savanna, an increase in ANPP with increasing woody plant cover was caused by an increase in woody ANPP, large enough to more than compensate for the decrease in grass ANPP (Reich *et al.* 2001). In contrast, ANPP was reduced with shifts from grass to woody plant dominance in desertified systems of New Mexico (Huenneke *et al.* 2002). In our study, the shift from herbaceous to woody dominance resulted in an increase in woody ANPP and in a decrease in herbaceous ANPP as well as in a significant change in the community ANPP. The increase in shrub ANPP was mostly accounted by *C. ladanifer* and it was in the plots dominated by this species ('advanced succession' plots) that ANPP significantly increased. *C. ladanifer* is a pioneer species that colonises degraded areas and forms one of the first stages of succession of woody communities (Correia 2002). Under unfavourable conditions, *C. ladanifer* successfully competes with other species and quickly spreads over recently disturbed areas. This species can grow fast and attain large amounts of biomass in a short amount of time (Nuñez *et al.* 1989). Patón *et al.* (1998) found a production of 170 g m^{-2}, in natural communities, in 2 year-old *C. ladanifer*, and Simões (2002), reported values ranging from 420.6 to 538.8 g m^{-2} for photosynthetical

active components for this species. In the studied system, the early ruderal herbaceous communities were replaced by pioneer shrub species that have themselves high growth rates. Therefore the increase in ANPP may not be a surprising result in these systems.

CONCLUSIONS

Our study showed an increase in total above-ground biomass with abandonment time indicating a positive effect of the shift to woody vegetation on total above-ground biomass. Total ANPP was significantly higher in 'advanced succession' than in 'grazing' while herbaceous ANPP decreased indicating a negative effect of increase in shrub cover on herbaceous ANPP. The decrease in herbaceous ANPP was compensated by the increase in shrub ANPP, which was greatly due to the high ANPP of *Cistus ladanifer*.

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CHAPTER 4

EFFECTS OF LAND USE CHANGE ON PLANT LITTER DECOMPOSITION IN AN EXTENSIVE AGRO-PASTORAL SYSTEM: RELATION TO LITTER CHEMISTRY AND PLANT FUNCTIONAL TRAITS

INTRODUCTION

Recycling of carbon and nutrients during decomposition is an important ecosystem process. It has a major control over the carbon cycle, nutrient availability, and, consequently, plant growth and community structure (Bardgett and Shine 1999). In ecosystems where nutrients are limited, such as those of the Mediterranean type, the amount of plant material returning to the soil and the rate of litter decomposition are particularly critical factors (Fioretto *et al.* 2003). Litter decomposition is controlled by abiotic factors, such as climate and soil chemical and physical properties, and by biotic factors, such as litter quality and the nature and abundance of the decomposer organisms (Coûteaux *et al.* 1995, Chapin III *et al.* 2002). Although climate is the factor that most strongly influences decomposition, within a particular climatic region, litter quality is the factor exerting the strongest control on litter decomposability (Aerts and Chapin 2000). Decay is controlled by a wide variety of chemical properties of litter including its nitrogen concentration, carbon to nitrogen ratio, phosphorus concentration or carbon to phosphorus ratio, lignin concentration or lignin to nitrogen ratio, cellulose and hemicellulose concentration (Melillo *et al.* 1982, Aerts and Chapin 2000, Cortez *et al.* 1996). Some studies report strong correlations between litter initial nitrogen concentration, C:N ratio, or lignin or lignin:N ratio, and the decomposition rate (*e.g.* Jamaludheen and Kumar 1999, De Angelis *et al.* 2000, Melillo *et al.* 1982, Cortez *et al.* 1996). Litter initial phosphorus concentration has also been pointed out to be well correlated to decomposition rate (Aerts *et al.* 2003). However, the chemical properties that best predict decomposition can vary across groups of species (Eviner and Chapin 2003) and climatic region. When Mediterranean adapted vegetation is considered, physical properties are of great importance and leaf toughness has been considered the best predictor by Gallardo and Merino (1993) for Mediterranean species.

In addition to the effects of litter quality, the presence of growing plants significantly alters the decomposition dynamics through effects on microbial activity and in soil temperature and

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moisture, both increasing and decreasing rates of decomposition (Eviner and Chapin 2003). Because plant species can differ in their effect on ecosystem structure and function, shifts in vegetation composition due to land use change can have strong effects on ecosystem processes such as nutrient cycling, primary productivity and trophic transfer (Pérez-Harguindeguy *et al.* 2000).

We investigated the effects of land use change on a key ecosystem process, decomposition, along a gradient of decreasing land use intensity in an agro-pastoral system in Alentejo, Southern Portugal, where, as a consequence of the rapid industrial and socio-demographic changes of the last decades, large areas of former arable land are being abandoned. At the vegetation level, the major consequence of abandonment is the change in plant composition and community structure. Abandonment, in this system, leads to the replacement of annual herbaceous communities with shrub communities, frequently dominated by one or few species (see chapter 2). Previous studies have shown that decomposition patterns are modified during succession and that these changes may be associated with species replacement during succession (Aber and Melillo 1982, Pardo *et al.* 1997). This vegetation change might be expected to coincide with slower decomposition rates as a consequence of the decline in litter quality as later-succession species replace earlier ones (Cornelissen *et al.* 1999). While assessing the effects of land use change on litter quality and litter decomposition, we tested the hypothesis that decomposition tends to slow down with abandonment, as woody species, richer in lignified structures replace herbaceous species.

Recent works have demonstrated strong associations between decomposition and leaf chemical composition (Wardle 2002) and physical properties (Cornelissen *et al.* 1999, Pérez-Harguindeguy *et al.* 2000). Decomposition of herbaceous communities was correlated to community specific leaf area, leaf dry matter and nitrogen content in a study of land abandonment in Mediterranean Southern France (Garnier *et al.* 2004). We tested whether

these traits of leaving leaves were also related to litter decomposition at sites with a high woody component and where important changes in life form occur, such as the ones in the present study.

METHODS

Study area

The study site (Monte do Vento, Mértola) is located in the Southeast of Portugal, at about 37°48'21.72" N and 7°40'44.96" W. The area has the typical winter-wet, summer-dry pattern of a Mediterranean type climate with little if any precipitation from June to September. Mean annual rainfall is around 438 mm and mean annual temperature of 16.8° C. The variation in temperature and precipitation during the study period is shown in Fig. 1. Most of the rainfall was concentrated on the first three months of the study and the mean annual rainfall was lower than the normal mean for a period of 30 years presented above.

At the study site, we selected three land use categories to represent a decrease in land use intensity. The first category, 'grazing', is used for extensive grazing by sheep (0.99 CU/ha). The two other categories, 'intermediate succession' and 'advanced succession', were abandoned, 10 to 15 years and 20 or more years ago, respectively. Within each land use category three permanent plots were set. Please refer to chapter 1 of this dissertation for a more detailed description of the study site and land use categories.
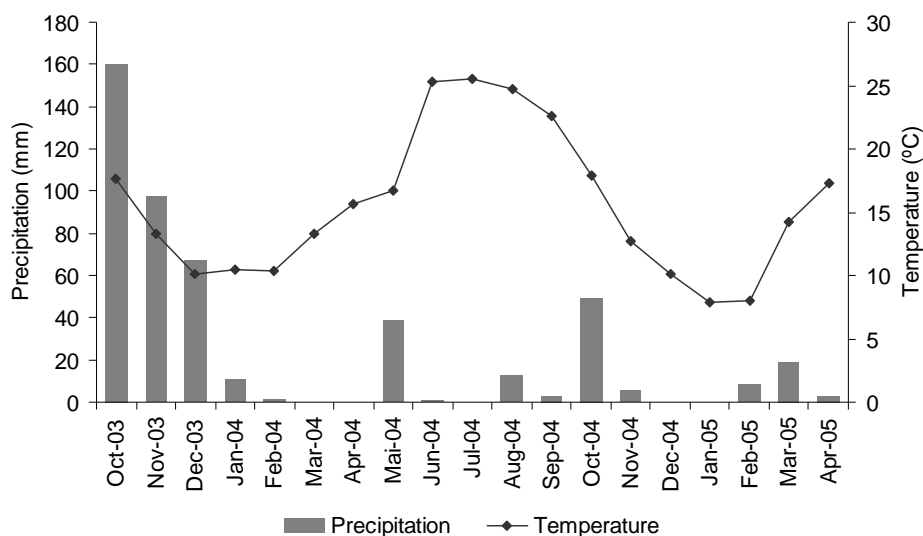
Figure 1 - Temperature and precipitation during the study period (October 2003 – April 2005), Mértola meteorological station.

Characteristics of plant communities and species traits

The three following traits, previously shown to relate to field age and litter mass loss (Garnier *et al.* 2004) and also tested for Mediterranean herbaceous communities by (Cortez *et al.* 2007), were measured: specific leaf area (SLA), leaf dry matter content (LDMC) and leaf nitrogen content (LNC). In addition to these traits we also measured leaf carbon content (LCC), leaf phosphorus content (LPC) and calculated leaf C:N ratio. These traits were measured following standardized protocols (Garnier *et al.* 2007, Cornelissen *et al.* 2003). Leaf nitrogen content, LCC and LPC were obtained from the analysis of a composite sample from the 5 individuals collected within each plot. Total amounts of carbon and nitrogen of leaves were determined with CHN elemental analyser (Carlo Erba elemental analyser, Model EA1108; CEFE – CNRS, Montpellier, France). Total amount of phosphorus was determined by a colorimetric method adapted from Murphy & Riley (1962). Samples were reduced to ashes at 500ºC for 3h and the ashes dissolved in 5ml cloridric acid. The resulting solution was adjusted to pH of 5 with NaOH solution and later to a total volume of 25 ml with bi-distilled

water. Finally, 4ml of the colorimetric reagent (ascorbic acid – ammonium paramolibdate complex) were added and the absorbance of the samples obtained at 882 nm in a spectrometer Jenway 6100. Please refer to chapter 2 for methodology regarding SLA and LDMC. Data on plant functional traits was collected for the most abundant species (see chapter 3 and Appendix 1).

Community-aggregated traits (hereafter community-trait) were calculated from species abundance and plant traits (Garnier *et al.* 2007). Please refer to chapter 2 for a more detailed description.

Litter decomposition

Litter decomposition studies were carried out using the litterbag technique as described in (Garnier *et al.* 2007). At the time of major peak of senescence, litter of all plant parts of all vascular plants species (in 'advanced succession' only shrub species were sampled) was collected in the proportions in which it is naturally shed in the plots. Coarse woody litter (>5 mm diameter), live biomass, substantially decomposed material, and seeds were excluded. Litter material was cut up into 5 cm lengths, in order to enable long leaves and stems to fit inside the litterbags and to get a representative mixture of the different parts and species in each litterbag. Litter was dried at room temperature for 3-4 days. A standard 1-mm mesh (Northern Mesh, Oldham, UK) was used to build flat bags of about 10 x 10 cm. The bags were filled with litter portions of 2g (±0.1).

Incubation was initiated in the beginning of October 2003. Litterbags containing community litter were incubated, at the soil surface, in the plot from which they came (Fig. 2). Four harvests were made after 3, 6, 12 and 18 months. The litter bag contents were carefully cleared of any extraneous plant material, soil animals, and soil aggregates, dried at 60ºC for 3 days and weighed.

Figure 2 - Example of the placement of the litter bags in the field.

To test the influence of plot local conditions on litter decomposition, samples of the same litter type, hay[2] (hereafter, standard litter), were incubated in each of the sampled plots. The samples were treated as described above.

To test the influence of litter quality, an additional decomposition experiment was carried out under standard condition in a microcosm at the 'Centre d'Ecologie Fonctionnelle et Evolutive' (CEFE), CNRS (Montpellier, France), as part of the objectives of project VISTA. Data was obtained from the project VISTA data set. For description of the methodology, please refer to Garnier (2007).

Litter quality:

A subset of the litter samples representative of the litter collected in each plot were ground to pass through a 1 mm mesh in a cyclone mill (Cyclotec Sample mill 1903), and scanned by near infrared reflectance spectrophotometer (NIRSystem 6500). Calibrations between initial

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[2] Hay was chosen as common litter type to all sites of Project VISTA.

litter spectral data and litter decomposability were calculated using cross-validated partial least square method. Four indicators of litter chemical quality were determined from initial litter spectral data at the CEFE, CNRS (Montpellier, France), following the method described by Joffre *et al.* (1992) and Gillon *et al.* (1999): nitrogen, lignin, cellulose and hemicellulose. In addition, three indices of litter chemistry, relating to the proportions of labile and non-labile compounds in the litter, were calculated (Cornelissen *et al.* 2004, Gillon *et al.* 1994, Cortez *et al.* 1996): the lignin:N ratio, the total fibre content (LCH = lignin + cellulose + hemicellulose), and the hollocellulose (cellulose + hemicellulose) to holocellulose + lignin ratio (HLQ). Total C was estimated as 50% of the ash-free biomass (Gallardo and Merino 1993, Schlesinger 1977).

Data analysis

Mass loss over time was fitted to a simple exponential model that assumes that a constant fraction of the mass is lost per unit time (Olson 1963) given by:

$$Ln\left(\frac{Mt}{M_0}\right) = -kt$$

where Mt is the mass at time t and M_0 is the original mass, and decomposition rates are given as fractional mass lost per year.

Mean litter mass loss values from each plot and harvest were calculated, and analysis performed treating each of the three plots in each land use category as a replicate. Single-factor ANOVA was used to test for differences in initial litter quality. Differences in litter mass remaining were tested using two-way ANOVA followed by the Tukey test. To test for significant differences between slopes of the regression functions we used the procedure described by Zar (1996) followed by a Tukey multiple comparison test to determine differences between slopes. The relationships between initial litter quality and litter mass

remaining and decay rate were assessed using Pearson's correlation coefficient. All statistical analyses were performed with SPSS 14.0 for windows (SPSS, Inc. Chicago, IL).

RESULTS

Litter mass loss and decomposition rate

A rapid initial phase of litter mass loss was observed for all litter types (Fig. 3). Litter mass remaining after 3 months of incubation was 81.43%, 71.92%, and 73.00%, in 'grazing', 'intermediate', and 'advanced succession' land use categories, respectively. Values for control litter were lower: 71.85%, 65.84% and 61.72% in 'grazing', 'intermediate', and 'advanced succession' land use categories, respectively.

Mass loss from community litter was significantly different among land use categories at the 6 and 18 months harvest and non-significant at the 3 and 12 months. Litter mass loss at the 6 month harvest was higher in abandoned plots than in grazed plots while, at the end of the experiment, bags in grazed plots had lost more weight than bags in 'advanced succession' plots. Litter mass loss in 'intermediate succession' land use category was not different from the other two categories. The mass loss of the standard litter only differed significantly at the 6 month harvest and as for community litter, mass loss was higher in abandoned than in grazed plots.

The Tukey test analyses showed a significant effect of land use category on litter decomposition rate. For the overall 18 month period (Table 1), 'grazing' land use category showed a significantly higher decomposition rate when compared to the other land use categories. Decay rates determined for 'advanced succession' land use category are in the range of values found for other Mediterranean evergreen species. Gallardo and Merino (1993) found decay rates between -0.14 and -0.50 year^{-1} after two years of decomposition for species

of *Cistus, Halimium* e *Quercus*. The decay rate at 6 months also differed significantly among land use categories but contrary to the overall results, 'grazing' land use category showed a significantly lower decay rate (Table 1). Litter from 'intermediate' and 'advanced succession' decomposed faster in the first 6 months of incubation and slower during the remaining period when compared to 'grazing' land use category (Table 1).
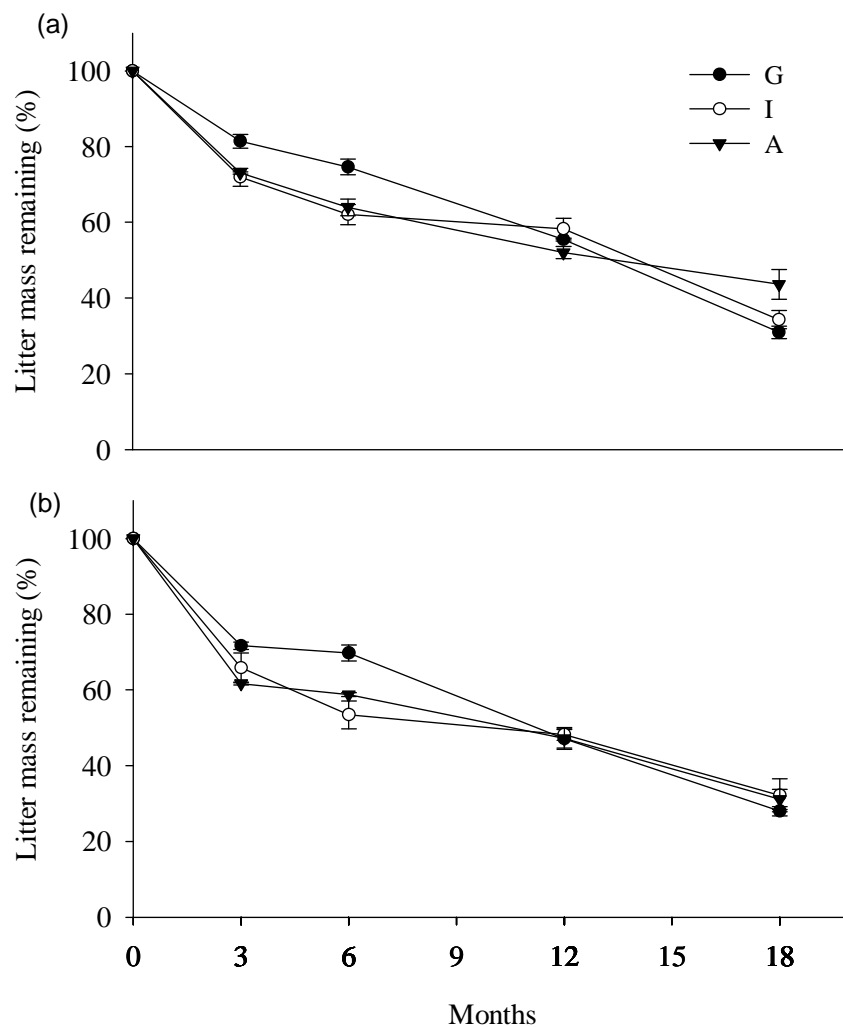

Figure 3 - Litter mass remaining (%) at different times from litterbags containing: (a) – community litter incubated *in situ*, (b) – standard litter incubated *in situ* (control litter). Mean values (± s.e.) are shown for the three replicate plots per land use category. G – grazing, I – intermediate succession, A – advanced succession.

Decomposition in microcosm showed decreasing decay rates with abandonment time, with 'grazing' land use category having highest decay rate and 'advanced succession' the lowest (F = 7.43, p < 0.05). 'Intermediate succession' was not different from the other land use categories.

Table 1 - Decomposition rates (year^{-1}) from community and standard litter in the different land use categories: G – grazing, I – intermediate succession, A – advanced succession. Values calculated from an exponential model at different collection dates.

	Decay rate					
	6 months		After 6 months*		Overall period	
	Community	Control	Community	Control	Community	Control
G	-0.607 [b]	-0.728 [a]	-0.869 [a]	-0.966 [a]	-0.732 [a]	-0.847 [b]
I	-0.963 [a]	-1.296 [b]	-0.434 [b]	-0.435 [b]	-0.578 [b]	-0.657 [a]
A	-0.914 [a]	-1.066 [a,b]	-0.443 [b]	-0.676 [b]	-0.575 [b]	-0.707 [a,b]

*considering mass remaining at 6 months as starting point

Litter initial quality

Initial chemical composition of litter is presented in table 2. Litter from 'grazing' land use category showed significantly lower values of Nitrogen (N) and was significantly richer in cellulose and hemicellulose when compared to 'intermediate' and 'advanced succession' land use categories. Lignin and carbon (C) content, as well as, lignin:N and C:N rations were not significantly different among land use categories. The lack of significant differences in lignin was likely due to large variation between replicas. Litter phosphorus content increased while LCH and HLQ decreased from 'grazing' to 'advanced succession'.

Table 2 - Initial chemical composition of species mixtures from the three land use categories. Litter chemical parameters: N- nitrogen, C- carbon, P- phosphorus, C:N- carbon to nitrogen ratio, Cell- cellulose, hemi- hemicellulose, lign- lignin, LCH- total fibre content, HLQ- holocellulose to holocellulose + lignin ratio. Land use categories: G- grazing, I- intermediate succession, A- advanced succession. Values are means ± 1 s.e. (n=3). The last row gives the results of the one-way ANOVA: n.s.- non significant, * p<0.05, ** p<0.01, *** p<0.001. Values with different superscript letter are significantly different (significance of Tukey-test given at p<0.05)

	N (mg g^{-1})	C (mg g^{-1})	P (mg g^{-1})	C:N (mg g^{-1})	Cell (mg g^{-1})	Hemi (mg g^{-1})	Lign (mg g^{-1})	Lign:N	LCH	HLQ
G	4.81± 0.71[a]	478.93 ± 2.20	0.39± 0.07[b]	104.36± 16.14[b]	326.02± 8.75[b]	263.64± 10.90 [b]	51.6± 16.20	10.1± 2.47	641.30± 4.00[a]	0.92± 0.03[a]
I	8.59± 0.51[b]	479.86 ± 0.13	0.56± 0.09[a,b]	56.29± 3.71[a]	164.62± 24.23[a]	133.42± 27.03 [a]	102.± 25.47	12.3± 3.93	400.33± 73.10[a,b]	0.75± 0.02[b]
A	9.25± 0.11[b]	478.00 ± 0.11	0.76± 0.2 [a]	51.68± 0.75[a]	149.16± 8.91[a]	95.759± 2.43 [a]	68.9± 8.36	7.45± 0.91	313.83± 7.69[b]	0.78± 0.03[b]
One-way Anova										
F	22.34**	0.30n.s.	7.00*	9.29*	46.79***	27.22**	2.03n.s.	0.82n.s.	15.94**	14.54**

Plant traits

Results from community-trait analysis showed significant differences in LDMC, SLA and LCC between land use categories (Table 3). Plots abandoned for longer (advanced succession) had species with higher community LDMC and LCC, and lower SLA. Community-LNC and LPC, as well as leaf C:N did not show significant differences among land use categories (Table 3).

Table 3 – Community-aggrgated traits in the three land use categories. Community-aggregated traits: SLA - specific leaf area, LDMC - leaf dry matter content, LNC- leaf nitrogen content, LCC- leaf carbon content, LPC- leaf phosphorus content, Leaf C:N- leaf carbon to nitrogen ratio. Land use categories: G- grazing, I- intermediate succession, A- advanced succession. Values are means ± 1 s.e. (n=3). The last row gives the results of the one-way ANOVA: n.s.- non significant * p < 0.05, ** p < 0.01, *** p < 0.001. Values with different superscript letter are significantly different (significance of Tukey-test given at p<0.05)

Land use	SLA $(m^2 kg^{-1})$	LDMC $(mg\,g^{-1})$	LNC $(mg\,g^{-1})$	LCC $(mg\,g^{-1})$	LPC $(mg\,g^{-1})$	Leaf C:N
G	24.14± 0.92[a]	222.10± 7.52[a]	23.26± 0.62	427.24± 0.92[a]	1.22± 0.07	18.39± 0.44
I	15.92± 0.22[b]	329.31± 10.00[b]	22.06± 1.40.	462.47± 1.01[b]	1.31± 0.003	21.11± 1.22
A	10.95± 1.21[c]	471.69± 5.06[c]	21.01± 1.35.	478.02± 0.81[c]	1.25± 0.007.	22.96± 1.60
One-way Anova						
F	56.59***	257.98***	0.916 n.s.	803.97***	1.024 n.s.	3.74 n.s.

Linking traits, litter chemistry and decomposition

Of the litter chemical parameters measured, cellulose, hemicellulose and LCH were positively related to litter decomposition rate of the overall study period both in the *in situ* experiment and in microcosm (Table 4). On the contrary, N and P were negatively related to decomposition rate. Considering the relationships between the chemical parameters measured and decomposition rate at other harvest times, none of the parameters was correlated with decay rate at the 12 month harvest. Nitrogen content was positively correlated with decay rate at the 3 and 6 month harvests while cellulose showed a negative correlation.

Table 4 - Relationships (Pearson's correlation coefficients) between decomposition of community litter under standard conditions (microcosm) or field and litter chemistry. Litter chemical parameters: N- nitrogen, C- carbon, P- phosphorus, C:N- carbon to nitrogen ratio, Cell- cellulose, hemi- hemicellulose, lign- lignin, LCH- total fibre content, HLQ- holocellulose to holocellulose + lignin ratio. Significance levels: n.s.- non significant, * p < 0.05, ** p < 0.01.

	microcosm	k3	K6	k12	K18
N	-0.717*	0.700*	0.677*	0.406	-0.742*
P	-0.749*	0.381 n.s.	0.334 n.s.	0.429 n.s.	-0.740*
C	0.600 n.s.	-0.234 n.s.	-0.067 n.s.	-0.232 n.s.	0.091 n.s.
C:N	0.546 n.s.	-0.597 n.s.	-0.612 n.s.	-0.317 n.s.	0.625 n.s.
Lignin	0.162 n.s.	0.382 n.s.	0.521 n.s.	-0.186 n.s.	-0.254 n.s.
Lignin:N	0.588 n.s.	-0.057 n.s.	0.116 n.s.	-0.432 n.s.	0.221 n.s.
Cellulose	0.789*	-0.776*	-0.741*	-0.409 n.s.	0.804**
Hemicellulose	0.808**	-0.681 n.s.	-0.657 n.s.	-0.452 n.s.	0.776*
LCH	0.872**	-0.682*	-0.621 n.s.	-0.419 n.s.	0.701*
HLQ	0.453 n.s.	-0.689*	-0.734*	-0.207 n.s.	0.701*

Several of the measured litter chemistry parameters were strongly related to the measured community-traits (Table 5). Litter N and P were negatively related to community-SLA, and positively related to community-LDMC and LCC. Litter cellulose, hemicellulose concentrations and LHC were positively related to community-SLA, and negatively related to community-LDMC and LCC.

There were clear links between litter decay rate and community-aggregated traits in the *in situ* experiment (Table 6). Community-LDMC and LCC were significantly and negatively related to decay rate at the end of the incubation period, both in *in situ* and under standard conditions while community-SLA showed a positive correlation with decay rate only under standard conditions.

Table 5. - Relationships (Pearson's correlation coefficients) between community-aggregated traits and litter chemistry. Community-aggregated traits: SLA - specific leaf area, LDMC - leaf dry matter content, LNC- leaf nitrogen content, LCC- leaf carbon content, LPC- leaf phosphorus content, Leaf C:N- leaf carbon to nitrogen ratio. Litter chemical parameters: N- nitrogen, C- carbon, P- phosphorus, C:N- carbon to nitrogen ratio, Cell- cellulose, hemi- hemicellulose, lign- lignin, LCH- total fibre content, HLQ- holocellulose to holocellulose + lignin ratio. Significance levels: n.s.- non significant, * $p < 0.05$, ** $p < 0.01$.

	SLA	LDMC	LNC	LCC	LPC	C:N
N	-0.846*	0.853**	-0.276 n.s.	0.930**	0.195 n.s.	0.574 n.s.
P	-0.740*	0.884**	-0.375 n.s.	0.800**	-0.125 n.s.	0.595 n.s.
C	0.144 n.s.	-0.146 n.s.	-0.166 n.s.	0.075 n.s.	-0.440 n.s.	0.124 n.s.
C:N	0.759 n.s.*	-0.765*	0.233 n.s.	0.855 n.s.	-0.142 n.s.	-0.519 n.s.
Lignin	-0.287 n.s.	0.122 n.s.	-0.230 n.s.	0.342 n.s.	-0.286 n.s.	0.294 n.s.
Lignin:N	0.176 n.s.	-0.339 n.s.	-0.058 n.s.	-0.166 n.s.	0.198 n.s.	-0.033 n.s.
Cellulose	0.88**	-0.845**	0.335 n.s.	-0.942**	0.349 n.s.	-0.612 n.s.
Hemicellulose	0.892**	-0.897**	0.389 n.s.	-0.942**	-0.264 n.s.	-0.657 n.s.
LCH	0.865**	-0.885**	0.328 n.s.	-0.912**	-0.260 n.s.	-0.600 n.s.
HLQ	0.739*	-0.636 n.s.	0.376 n.s.	-0.808**	-0.378 n.s.	-0.583 n.s.

Table 6 - Relationships (Pearson's correlation coefficients) between decomposition of community litter under standard conditions (microcosm) or field and community-aggrgated traits: SLA - specific leaf area, LDMC - leaf dry matter content, LNC- leaf nitrogen content, LCC- leaf carbon content, LPC- leaf phosphorus content, Leaf C:N- leaf carbon to nitrogen ratio. Significance levels: n.s.- non significant * $p < 0.05$, ** $p < 0.01$.

	microcosm	k3	k6	k12	K18
SLA	0.789*	-0.751*	-0.809*	-0.599 n.s.	0.660 n.s.
LDMC	-0.836**	0.609 n.s.	0.615 n.s.	0.647 n.s.	-0.688*
LNC	0.206 n.s.	-0.186 n.s.	-0.420 n.s.	-0.266 n.s.	0.248 n.s.
LCC	-0.804*	0.793*	0.801**	0.542 n.s.	-0.766*
LPC	-0.325 n.s.	0.435 n.s.	0.475 n.s.	-0.123 n.s.	-0.179 n.s.
C:N	-0.445 n.s.	0.444 n.s.	0.633 n.s.	0.458 n.s.	-0.444 n.s.

DISCUSSION

Litter mass loss

All litter types showed rapid initial litter mass loss. After 90 days litter mass loss ranged from about 18% in 'grazing' land use category to 28% in 'advanced succession' land use category. The rapid initial mass loss may be attributed to initial leaching of readily soluble components of the litter. This initial mass loss coincided with autumn and early winter and with the highest rainfall amount registered in the entire study period (Fig. 1). Under Mediterranean conditions, large litter mass losses have been reported to occur during the favourable conditions of autumn and, end of winter and beginning of spring (Gallardo and Merino 1993, Moro and Domingo 2000, Simões 2002). Additionally, rainfall directly influences litter breakdown in initial decomposition stages through leaching of soluble compounds and indirectly through impacts on the microbes and fauna (Coûteaux *et al.* 1995). Lensing and Wise (2007) found that litter decay was 50% faster in high rainfall plots that in low rainfall plots, in a temperate deciduous forest. Simões (2002) also found higher mass loss to be associated with periods of higher rainfall in litter decomposition of two *Cistus* species in South Portugal. The author reported a mass loss of 20% for the first 65 days under precipitation values similar to those of the present study.

Litter mass loss of community litter at the end of the study period ranged from about 56% in 'advanced succession' to 69% in 'grazing'. These values are in the range of those reported by Simões (2002), Fioretto *et al.* (2003), and Gallardo and Merino (1993) in shrub communities (values ranging from about 20 to 50%) and by Cortez *et al.* (2007) for grasslands (around 60%) in Mediterranean areas.

Effects of litter chemistry and land use category on litter decomposition

The hypothesis that decomposition will tend to slow down with abandonment, as woody species replace herbaceous species, was partially confirmed by our results. Overall, there was an effect of land use category on litter decomposition as shown by higher decomposition rate of litter from 'grazing' when compared to 'intermediate' and 'advanced succession' plots. This seems to reflect the differences in the initial litter composition, which is supported by the results of the microcosm experiment. In accordance to *in situ* results for the entire incubation period, results from microcosms showed a decrease in decomposition rate with abandonment, indicating that litter quality is important in controlling litter decomposition (Coûteaux *et al.* 1995, Aerts 1997).

Several litter chemistry parameters were correlated with litter decomposition, both *in situ* and in microcosm and the amount of holocellulose was one of the main determinants of litter decomposition rate. Litter from 'grazing' land use category showed higher values of cellulose and hemicellulose, when compared to 'intermediate' and 'advanced succession' land use categories, and these were positively correlated with decomposition rate, both *in situ* and in microcosm. The chemical components of litter can be divided into three broad groups: soluble substances, insoluble polymer carbohydrates (holocellulose) and insoluble phenolic substances (mostly lignin). Isolated holocellulose (cellulose + hemicellulose) is easily degraded by microorganisms (Berg *et al.* 1984). The decomposition rate of cellulose and hemicellulose is generally higher than that of lignin (Fioretto *et al.* 2005, Berg and McClaugherty 2008). However, when all unshielded holocellolose is decomposed only lignin incrusted holocellulose and lignin remain, and, at this point, both components are degraded at the same rates because they are so well mixed in the fibre structure that they can not be degraded separately (Berg and McClaugherty 2008). Thus, at this stage, lignin rules the litter decomposition rate (Berg and Laskowski 2006a). The larger contents of cellulose and hemicellulose relative to lignin in litters

from 'grazing' suggest that a larger amount of unshielded holocellulose was available, and likely played a role in determining the higher decomposition rate of these litters.

Litter lignin concentration and the C:N and lignin:N ratios, that are frequently used as indicators of decomposition, were not significantly related to decomposition rates in our study. This is consistent with the results from a review of 96 temperate zone decomposition experiments where only a relationship with litter N concentration was found (Aerts 1997). It is not consistent with previous studies from Mediterranean areas where good correlations were found between litter decomposition and C:N ratio (Cortez *et al.* 2007, Moro and Domingo 2000) or lignin:N (Gallardo and Merino 1993).

The pattern of litter mass loss was not consistent throughout the time of the study. Litter decomposition in 'intermediate' and 'advanced succession' was faster in the first 6 months and slower thereafter when compared to 'grazing' land use category. Litter decomposition is generally accepted to be divided in two parts: decomposition of labile litter constituents (hydrosolubles, non-lignified cellulose), which is controlled largely by the stimulating effects of nutrient concentration (mainly nitrogen), and decomposition of lignified carbohydrates, that depends on the initial lignin content (Berg and Laskowski 2006b, Couteaux *et al.* 1998). Soluble substances and labile compounds are rapidly degraded in early stages of decomposition by fast growing microorganisms that may require a high concentration of nitrogen (Fioretto *et al.* 2005). High initial N content may lead to high initial decay rates, but to low long-term litter decay (Berg *et al.* 1996, Aerts 1997) because in a high N environment, the growth of slow-growing fungi, able to decompose lignin is reduced due to competition with fast growing microbes (Fioretto *et al.* 2005, Berg *et al.* 1996). Initial litter nitrogen concentration was higher in 'intermediate' and 'advanced succession' when compared to 'grazing' and it was positively related to decomposition rate in early stages. Moreover, even though there were no significant differences in control litter at the end of the study period, for

the first six months it followed a pattern similar to that of community litter, decomposing faster in abandoned than in grazed plots. This suggests that, at least during this period, there was an effect of the site microenvironment. The rates of decomposition are known to be correlated to soil moisture and temperature regimes and the chemical properties of the soil and litter, especially the initial N concentration, C:N ratio, in the litter and surrounding soil (Hobbie 1996, Chadwick *et al.* 1998). The decomposition of control litter in abandoned areas may have been enhanced by higher litter N of community litter. Additionally, the shift from herbaceous to shrub dominated communities with consequent changes in species composition and community structure may have resulted in different effects on soil moisture, temperature and root exudates thereby influencing decomposition differently (Eviner and Chapin 2003). However, if this effect was present, as the results for the first 6 months may suggest, it was overcome by the effect of litter quality in the second half of the decomposition period. Further studies are needed in order to better understand the effects of litter quality and microclimate on litter decomposition at the study site.

Linking traits, litter chemistry and decomposition

The replacement of species during the course of succession led to changes in functional traits of the plant community with the replacement of species with high SLA and low LDMC and LCC with species having opposite traits. At the single species level, LDMC has been related to lignin in litter and to decomposition rate (Kazakou *et al.* 2006). At the community level, LDMC and SLA were found to correlate to litter carbon and nitrogen concentration, hemicellulose and C:N ratio, in Mediterranean herbaceous communities (Cortez *et al.* 2007). Also at the community level, LDMC was found to correlate positively to lignin, LCH, and lignin:N and negatively with cellulose, hemmicellulose and HQL along gradients of decreasing land use intensity in a study across 11 sites with different climate (Fortunel *et al.* in press). In

the same study, LDMC and LCC were found to be negatively correlated to decomposition rate (Fortunel *et al.*). Slower decomposition rates were also found in Mediterranean herbaceous communities composed of species with high LDMC (Garnier *et al.* 2004, Cortez *et al.* 2007). In the present study, LDMC and SLA were linked to key chemical litter quality indices, such as nitrogen and phosphorus concentration, C:N ratio, cellulose and hemicellulose and the LCH index. Regarding the correlation between leaf traits and decomposition rate, LDMC and LCC were negatively related to decomposition rate of the all study period. Leaf dry matter content is a strong indicator of the relationship between the different structural components of the leaf that make litter more or less decomposable, i.e., it is and index of the amount of mesophyll vs. structural compounds (lignin, cellulose, hemicellulose; Garnier and Laurent 1994). Our results confirm that also at the community level, traits more related to decomposition are those more strongly linked to physical attributes of the leaves, such as LDMC, as found by Kazakou *et al.* (2006) at the species level. This is interesting considering that community litter, particularly that containing herbaceous species, was composed of a considerable quantity of stems, which likely differ from leaves in their composition.

CONCLUSIONS

Overall, our results are in line with a number of studies that have identified a link between increasing successional age and the presence or abundance of plant species which produce low quality litter (Wardle *et al.* 1997, Garnier *et al.* 2004, Kazakou *et al.* 2006).

Our results are also in agreement with previous studies that indicate that that shifts in plant life form alter ecosystem litter chemistry, which in turn, control decomposition rates and soil organic matter quantity and quality. Shrub litter was found to be higher than herbaceous litter in nutrient content, especially nitrogen, which seemed to favour higher initial decomposition rates but lower decomposition rate in the longer term. Shrubs also contribute

with woody litter, richer in lignin and secondary compounds that retard decomposition, and may play a role in increasing pools of slowly decomposing soil carbon (Gill and Burke 1999).

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FINAL REMARKS

The studied agro-pastoral system is the result of centuries of human management and its structure is dependent on human practices and management. Similarly to the Montado, it likely originated from mixed forests of holm and cork oak and other species with persistent leaves (Capelo and Catry 2007). Control of invasion by shrub species is usually achieved by clearing and ploughing even though grazing also contributes to control shrub invasion (Fernández Alés *et al.* 1993, Joffre *et al.* 1999). When these cease, it is invaded by Mediterranean matorral species (Fernández Alés *et al.* 1993). The results of our study confirm these earlier findings. Grazed plots were composed mostly of annual herbaceous species, whose number and abundance decreased in abandoned plots, and of few disperse holm and cork trees. On the contrary, shrub cover increased with abandonment. In 'intermediate succession' plots the dominant shrub species were chamaephytes (mainly *Lavandula stoechas*) while 'advanced succession' plots were dominated by nanophanerophytes, in particular *Cistus ladanifer*. This change in life form dominance was accompanied by a decrease in plant species richness, which was mostly a consequence of the decrease in the number of herbaceous species.

The dominance of *Cistus ladanifer* after 20 years of abandonment is an interesting aspect to point out in the present study. The existence of communities dominated by species from the genus *Cistus* has been reported to occur following abandonment of agro-pastoral practices (Correia 2002). It has been suggested that such communities may represent a case of arrested succession, *i.e.* succession is strongly delayed or stopped (Acacio *et al.* 2007). In the studied system, and in the absence of human management and other disturbances, the expected pathway of natural succession would be through the gradual colonization of the understorey by pioneer shrubs followed by other shrub communities mixed with oak natural regeneration leading to an oak forest (Correia 1998). However, Mediterranean ecosystems have a long history of human management and regeneration of original vegetation types may not occur. In

these cases, persistent alternative stages of succession may occur (Westoby *et al.* 1989, Scheffer *et al.* 2001). A recent study of the regeneration of cork oak showed that there are several recruitment limitations (high levels of seed predation, low seedling survival) and that seedling recruitment is severely limited in *Cistus* shrublands (Acacio *et al.* 2007). *Cistus ladanifer* is known to have allelopathic affects inhibiting the seedling germination and growth of several plant species (Chaves and Escudero 1997, Herranz *et al.* 2006). This may explain why after 20 years of abandonment there is a strong dominance of *Cistus ladanifer*. On the other hand, *Genista hirsuta*, which is also present in 'advanced succession' plots, has been shown to have facilitative effects on the recruitment of holm oak (Smit *et al.* 2008). Further studies including plots abandoned for longer times and an assessment of possible holm and cork oak regeneration would be necessary in order to understand whether successional pattern at the study site could progress towards its expected climax vegetation.

The change in vegetation composition was followed by a change in plant functional traits (Fig. 1). Both at the species and community level, changes in plant functional traits with abandonment time pointed to the replacement of small, short-lived and generally fast growing species (high SLA and low LDMC), acquiring external resources rapidly, with medium and tall shrub species, which grow slower (low SLA and high LDMC), and tend to conserve internal resources more efficiently (Garnier *et al.* 2004a). Changes in trait values with abandonment time may be due to the replacement of species with different trait values, to changes in trait values within a species, or both (Garnier *et al.* 2004b). Here, changes in trait values were mostly due to species replacement because there was a high species turnover from 'grazing' to 'advanced succession'.

There is no universal functional group classification. It often depends on the aim and scale of the study and the ecosystem process or environmental factor of interest (Diaz and Cabido 2001). Here we searched for a functional group classification in response to abandonment. By

combining two plant functional traits, life form and SLA, we found several plant functional groups in response to abandonment. Therophytes with medium SLA were the dominant functional group in grazed areas, while nanophanerophytes with medium or low SLA were associated with later phases of abandonment. At intermediate stages of succession the dominant group was chamaephytes with medium SLA but functional diversity was highest, as all the groups, except hemicriptophytes with medium SLA, were represented.
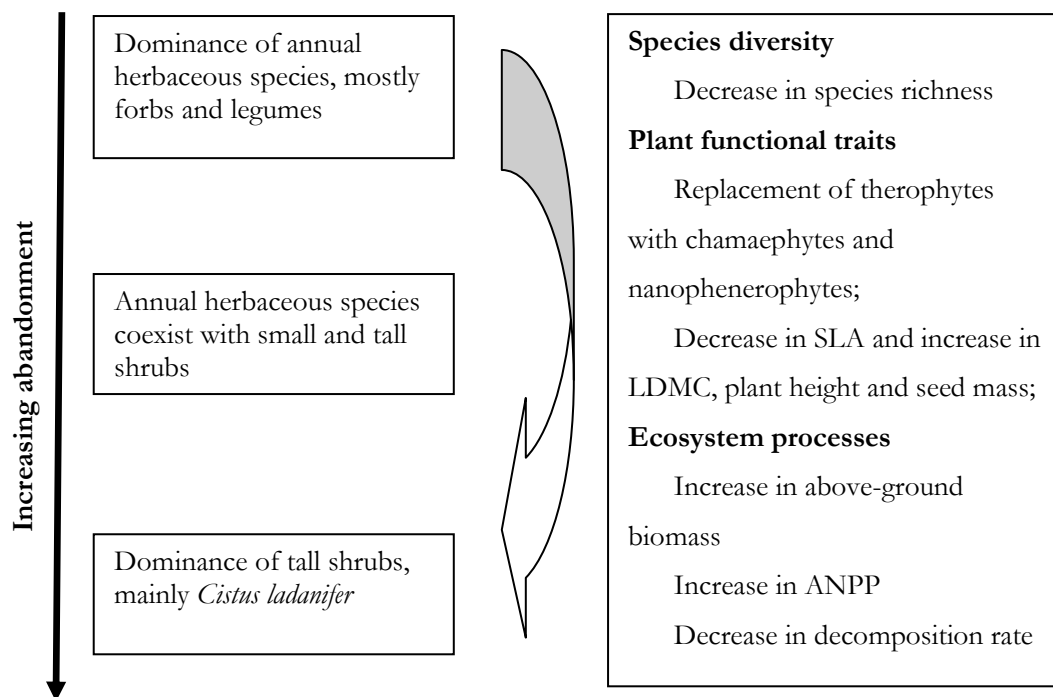

Figure 1. Summary of changes in vegetation, plant species richness, plant functional traits and ecosystem processes as a consequence of abandonment in the studied area.

The shift from herbaceous to shrub dominated communities resulted in changes in above-ground biomass and ANPP. Herbaceous above-ground biomass and ANPP decreased with abandonment, as woody cover increased, which is consistent with results from previous studies (Karakosta and Papanastasis 2007, Zarovalli *et al.* 2007). Total (herbaceous and shrubs)

above-ground biomass and ANPP increased with increasing shrub cover, particularly with the increase in the cover of *Cistus ladanifer*.

Shifts in land use and plant species composition can influence decomposition by a number of mechanism, including changes in the quality of the litter produced and influences on the temperature and moisture regime at the soil surface (Eviner 2004). Previous studies have indicated that shifts in plant life form alter total-ecosystem litter chemistry (*e.g.* Dorrepaal *et al.* 2005, Quested *et al.* 2007). Litter chemistry, in turn, controls decomposition rates and soil organic matter quantity and quality (Coûteaux *et al.* 1995). Overall, results showed that the shift from herbaceous to shrub dominated communities lead to a decrease in decomposition rate. Results also showed that the changes in community litter decomposition with abandonment were primarily driven by changes in litter quality even though an effect from possible differences in microclimate may also have played a role since the pattern of litter mass loss was not constant throughout the study period. Aboveground plant structure may affect ecosystem processes through airflow, albedo, and water percolation patterns (Gill and Burke 1999). Further studies are necessary in order to understand the effects of shifts on vegetation structure on soil microclimate and micro-organisms activity and how this in turn influences litter decomposition, as well as to separate the effects of litter quality and microclimate.

Collecting information at the species and ecosystem levels allowed us to look at the links between plant traits and ecosystem processes. By weighing trait values by the relative abundance of the species in the community, these specific traits could be related with ecosystem level processes (Garnier *et al.* 2004b). As shown in Fig.2, higher LDMC was related to lower decomposition rates, higher soil carbon and organic matter content, higher biomass and ANPP, and all of these are associated to longer abandonment times and to the increasing abundance of *Cistus ladanifer*. This species has LDMC values that are higher and SLA values

that are lower than any of the other species measured (Appendix 3) and a high abundance in 'adavanced succession' plots (Appendix 1) having therefore a large influence on community-trait values and, likely, a strong influence on ecosystem processes. This species showed high ANPP (see Chapter 3) and is known to have a high biomass and nutrient turnover representing an important input of organic matter and nutrients into the soil (Simões *et al.* 2001, Correia 2002).
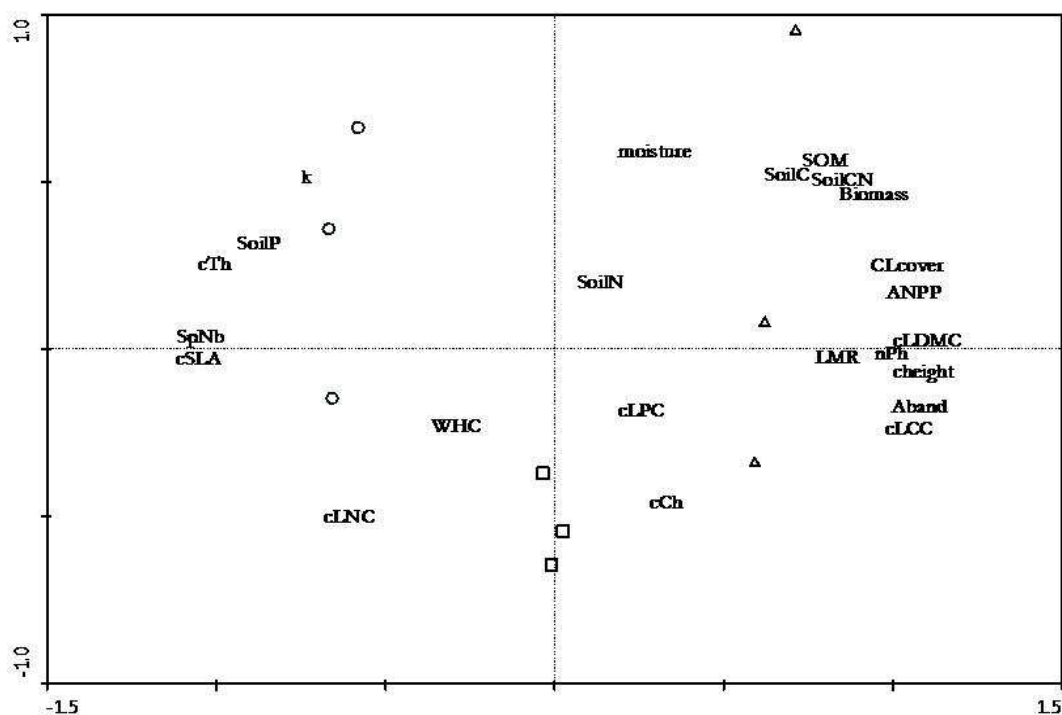

Figure 2 – Principal components analysis combining soil, community-aggregated trait and ecosystem data. Only the first two axes, which explain 77.4 % of the total inertia (67.7% is explained by the first axis). Analysis was carried out with Canoco 4.5 (TerBraak) on standardized data. Soil parameters: SoilP- soil phosphorus, SoilC- soil carbon, SoilN- soil nitrogen, WCH- soil water holding capacity, moisture- soil moisture, SOM- soil organic matter, SoilCN: soil carbon to nitrogen ratio; Community-aggregated traits: cTh- therophytes, cCh- chamaephytes, cPh- phanerophytes, cSLA- specific leaf area, cLDMC- leaf dry matter content, cLNC- leaf nitrogen content, cLCC- leaf carbon content, cLPC- leaf phosphorus content, cheight- plant height; ecosystem level data: SpNb- species number, biomass-above-ground biomass, ANPP- above-ground net primary productivity, k- decomposition rate, LMR-litter mass remaining; Others: Aband- abandonment time, CLcover- relative abundance of *Cistus ladanifer*. Symbols represent plots in the different land use categories: grazing (circles), intermediate succession (squares) and advanced succession (triangles).

The increase in the cover of woody species was also associated to higher soil carbon and organic matter content and higher soil C:N. Woodiness is particularly important in enhancing carbon sequestration because woody plants tend to contain more carbon, live longer, and decompose more slowly than smaller herbaceous plants (Díaz *et al.* 2005). Therefore the shift from herbaceous to shrub dominated communities may result in higher accumulation of C in these ecosystems, contributing to reduce CO_2 in the atmosphere. On the other hand, this shift represents an increase in the flammability (higher LDMC and canopy height) and amount of fuel (more biomass) which may increase the probability and severity of fires (Lavorel and Garnier 2002, Pausas 2004). Since the regeneration of *Cistus* species is generally favoured by fire (Pausas 1999b, Correia 2002), a positive feedback between fire and the maintenance of *Cistus* dominated shrublands may occur.

At the landscape level, the increasing abandonment may promote the appearance of large continuous areas of uniform flammable vegetation with the consequence of an increase in the number of fires, total surface burned and the distribution of areas affected by fire (Pausas 1999a). Additionally, the complete abandonment of agricultural uses implies the loss of a traditional landscape and many ecosystem functions as well as loss of habitat and species diversity (Plieninger *et al.* 2004, Moreira *et al.* 2005). On the other hand, and increase in shrubland areas may represent different potentials for other complementary uses such as hunting, beekeeping, collection of natural products or recreation, and are therefore important for the multifuntionality of the landscape (Pinto-Correia and Vos 2004) and services provided to humans. In order to maintain the multifunctionality of these landscapes and the services provided by these ecosystems a certain degree of management is necessary.

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APPENDICES

Appendix 1

Appendix 1. Species name, botanical family and mean relative cover (%) in each land use category. Information collected from literature or field observation on life form is also listed. Land use category: G – grazing, I – intermediate succession, A – advanced succession; Life form: Th – therophyte, Geo – geophyte, hCr – hemicryptophyte, Ch – chamaephyte, nPh – nanophanerophyte, Ph – phanerophyte. Species in bold represent the species for which trait values were obtained.

| Species | Family | Life form | Relative cover (%) | | |
			G	I	A
Agrostis curtisii	Gramineae	hCr	–	0.01	–
Agrostis pourretii	Gramineae	Th	**7.41**	0.27	0.13
Anagallis arvensis	Primulaceae	Th	0.29	0.14	0.05
Anarrhinum bellidifolium	Scrophulariaceae	hCr	0.14	0.13	–
Andryala integrifolia	Compositae	hCr	0.12	0.14	0.02
Anthyllis lotoides	Leguminosae	Th	**2.60**	0.93	0.22
Arisarum vulgare	Araceae	Geo	–	0.02	–
Avena barbata	Gramineae	Th	0.12	0.23	–
Biserrula pelecinus	Leguminosae	Th	**2.47**	0.23	0.11
Brachypodium distachyon	Gramineae	Th	**2.00**	**4.86**	1.52
Briza maxima	Gramineae	Th	0.09	0.25	0.08
Briza minor	Gramineae	Th	–	0.04	–
Bromus hordeaceus	Gramineae	Th	0.26	0.04	–
Calendula arvensis	Compositae	Th	0.13	–	–
Campanula lusitanica	Campanulaceae	Th	0.06	0.07	–
Carlina corymbosa	Compositae	Geo	0.47	**4.11**	0.79
Carlina racemosa	Compositae	Th	**6.07**	–	0.07
Centaurium erythraea	Gentianaceae	hCr	0.19	0.57	0.07
Cerastium glomeratum	Caryophyllaceae	Th	0.21	0.01	–
Chamaemelum mixtum	Compositae	Th	**10.92**	0.18	–
Cistus crispus	Cistaceae	nPh	0.09	–	–
Cistus ladanifer	Cistaceae	nPh	0.81	**10.33**	**52.39**
Cistus monspeliensis	Cistaceae	nPh	–	0.31	–
Cistus populifolius	Cistaceae	nPh	–	–	0.94
Cistus salvifolius	Cistaceae	nPh	–	–	0.22
Coleostephus myconis	Compositae	Th	0.09	0.26	–
Coronilla scorpioides	Leguminosae	Th	–	0.04	–
Crepis vesicaria	Compositae	–	0.09	0.08	–

Appendix 1. (cont.). Species name, botanical family and mean relative cover (%) in each land use category. Information collected from literature or field observation on life form is also listed. Land use category: G – grazing, I – intermediate succession, A – advanced succession; Life form: Th – therophytes, Geo – geophyte, hCr – hemicryptophyte, Ch – chamaephyte, nPh – nanophanerophyte, Ph – phanerophyte. Species in bold represent the species for which trait values were obtained.

Species	Family	Life form	Relative cover (%)		
			G	I	A
Crucianella angustifolia	Rubiaceae	Th	–	0.10	0.02
Cynara cardunculus	Compositae	H	0.05	–	–
Cynosurus echinatus	Gramineae	Th	–	0.04	–
Daphne gnidium	Thymelaeaceae	nPh	–	–	0.99
Daucus carota	Umbeliferae	hCr	–	0.30	0.24
Echium plantagineum	Boraginaceae	Th	0.22	0.07	–
Erodium moschatum	Geraniaceae	Th	0.06	0.05	–
Galactites tomentosa	Compositae	Th	0.14	0.36	–
Galium spp	Rubiaceae	Th	0.09	0.01	–
Gastridium ventricosum	Gramineae	Th	–	0.08	–
Gaudinia fragilis	Gramineae	Th	0.88	0.26	–
Genista hirsuta	Leguminosae	nPh	–	**15.64**	**22.49**
Genista triacanthos	Leguminosae	nPh	–	–	0.24
Geranium molle	Geraniaceae	Th	0.02	0.01	–
Helichrysum stoechas subsp. stoechas	Compositae	Ch	–	**1.64**	0.16
Holcus lanatus	Gramineae	hCr	**2.87**	0.38	–
Hyparrhenia hirta	Gramineae	hCr	–	0.02	–
Hypochoeris glabra	Compositae	Th	**5.49**	0.22	0.05
Jasione montana	Campanulaceae	Th	0.30	0.30	0.01
Juncus bufonius	Juncaceae	Th	0.08	0.11	–
Lathyrus angulatus	Leguminosae	Th	0.02	–	–
Lavandula stoechas subsp. luisieri	Labiatae	Ch	–	**32.06**	**13.38**
Lavandula viridis	Labiatae	Ch	–	**5.06**	**1.41**
Leontodon taraxacoides subsp. longirostris	Compositae	Th	**11.33**	**2.83**	0.57
Linaria amethystea	Scrophulariaceae	Th	–	–	0.02
Logfia gallica	Compositae	Th	**1.59**	0.29	0.08
Lolium rigidum	Gramineae	Th	0.47	–	–
Lotus subbiflorus subsp. castellanus	Leguminosae	Th	0.02	–	–

Appendix 1. (cont.). Species name, botanical family and mean relative cover (%) in each land use category. Information collected from literature or field observation on life form is also listed. Land use category : G – grazing, I – intermediate succession, A – advanced succession; Life form: Th – therophytes, Geo – geophyte, hCr – hemicryptophyte, Ch – chamaephyte, nPh – nanophanerophyte, Ph – phanerophyte. Species in bold represent the species for which trait values were obtained.

Species	Family	Life form	Relative cover (%)		
			G	I	A
Lotus conimbricensis	Leguminosae	Th	0.73	0.03	0.05
Olea europaea var. sylvestris	Oleaceae	Ph	–	–	0.02
Ornithopus compressus	Leguminosae	Th	**3.50**	0.52	0.15
Orobanche minor	Orobanchaceae	Th	0.81	0.17	–
Parentucellia viscosa	Scrophulariaceae	Th	**1.79**	**0.91**	0.05
Paronychia argentea	Caryophyllaceae	hCr	0.77	–	–
Paronychia cymosa	Caryophyllaceae	Th	0.06	0.19	–
Paronychia echinulata	Caryophyllaceae	Th	–	0.09	–
Phagnalon saxatile	Compositae	Ch	–	0.09	–
Plantago afra	Plantaginaceae	Th	0.34	–	0.02
Plantago coronopus	Plantaginaceae	hCr	**2.84**	0.14	–
Plantago lagopus	Plantaginaceae	H	0.03	–	–
Pulicaria paludosa	Compositae	Th	0.26	0.09	–
Quercus rotundifolia	Fagaceae	Ph	0.93	0.90	1.95
Quercus suber	Fagaceae	Ph	0.81	0.47	–
Raphanus raphanistrum	Cruciferae	Th	0.06	–	–
Rosmarinus officinalis	Labiatae	nPh	–	–	0.53
Rumex acetosella subsp. angiocarpus	Polygonaceae	H	**2.01**	0.12	–
Rumex bucephalophorus	Polygonaceae	Th	0.36	0.30	–
Rumex conglomeratus	Polygonaceae	H	0.09	0.02	–
Sanguisorba minor	Rosaceae	H	0.07	0.12	–
Scorpiurus vermiculatus	Leguminosae	Th	–	0.04	–
Senecio vulgaris	Compositae	Th	0.07	–	–
Silene gallica	Caryophyllaceae	Th	**0.91**	–	–
Spergularia capillacea	Caryophyllaceae	Th	0.30	0.02	–
Stachys arvensis	Labiatae	Th	0.07	–	0.02
Taeniatherum caput-medusae	Gramineae	Th	0.37	0.28	–
Teesdalia nudicaulis	Cruciferae	Th	0.07	–	–

Appendix 1. (cont.). Species name, botanical family and mean relative cover (%) in each land use category. Information collected from literature or field observation on life form is also listed. Land use category : Geo – grazing, I – intermediate succession, A – advanced succession; Life form: Th – therophytes, G – geophyte, hCr – hemicryptophyte, Ch – chamaephyte, nPh – nanophanerophyte, Ph – phanerophyte. Species in bold represent the species for which trait values were obtained.

Species	Family	Life form	Relative cover (%)		
			G	I	A
Tolpis barbata	Compositae	Th	**7.57**	**4.00**	0.47
Trifolium angustifolium	Leguminosae	Th	**1.50**	**1.64**	0.11
Trifolium arvense	Leguminosae	Th	**1.10**	**1.64**	0.02
Trifolium bocconei	Leguminosae	Th	**1.88**	**1.27**	0.02
Trifolium campestre	Leguminosae	Th	**2.82**	**1.96**	0.05
Trifolium cherleri	Leguminosae	Th	1.26	0.17	–
Trifolium glomeratum	Leguminosae	Th	0.78	0.28	–
Trifolium hirtum	Leguminosae	Th	0.09	–	–
Trifolium spumosum	Leguminosae	Th	0.02	–	–
Trifolium stellatum	Leguminosae	Th	0.41	0.04	–
Trifolium subterraneum	Leguminosae	Th	0.70	–	–
Trifolium tomentosum	Leguminosae	Th	0.05	0.02	–
Tuberaria guttata	Cistaceae	Th	0.23	0.17	–
Vulpia bromoides	Gramineae	Th	**5.42**	1.14	0.22
Vulpia geniculata	Gramineae	Th	0.65	0.02	–

APPENDIX 2

The original fourth corner method multiplies the presence/absence matrix **A** (k×m) of k species recorded at m sites with a matrix **B** (k×n) coding the species traits into n classes and a third matrix **C** (p×m) assigning the p environmental conditions to the m sites. The matrix product **D**=**CA'B** lists the frequencies at which each plant group occurs at a given treatment. These count data are not suitable for Chi-square testing, because the observations are not independent of each other (several species may occur at one site). A randomisation (null model) test is used instead of a classical test. Matrix **A** is randomised using a null model, and for each null community (**A**$_{per}$) a new matrix **D**$_{per}$ is computed (**D**$_{per}$=**CA**$_{per}$**'B**). For each cell in **D**, the frequency of containing a value greater than or equal to the associated cells in the set of **D**$_{per}$ is calculated. If an entry in **D**$_{per}$ is only rarely greater than or equal to the corresponding entries in **D**, the trait combination is thought to occur more often than expected by the null model, and is positively related to the treatment. Given a large set of permutations, this frequency is an estimator of the one- tailed probability (p-value) of **D**(cell)≥**D**$_{per}$(cell). If the p-value is below 0.05, the group is considered to respond to the associated treatment. Values higher than 0.5 indicate a negative association i.e. the plant group occurs less often than expected by the null model. In this case a (-) sign indicates the probability of generating a value less than or equal to the value in **D**.

Legendre *et al.* (1997) corrected their p-values to accommodate for the increased probability of committing a Type I error in the case of multiple simultaneous tests. We decided not to correct the p-values, because (i) each plant group will be compared individually against the occurrence of the same group in the null model and (ii) no indirect comparisons are made between different plant groups or treatments.

The extended fourth corner method gives a matrix of p-values for each plant classification. Subsequently an optimisation criterion is applied to find the most appropriate one. Such

criteria can be designed according to different objectives. One might aim at a small set of FRGs with a strong response of each FRG to all environmental conditions, at the expense of not identifying FRGs which may only have a significant response to some environmental conditions. On the other hand, a larger set of FRGs can result in more significant responses in total but fewer per FRG. The optimal set of FRGs is chosen in a two-step procedure. In the first step, the total response of all sets are compared with each other based on their p-values to test whether the combination of two adjacent groups into a single group leads to a similar or even better response for the combined group. In this case the larger group is discarded if the direction of response of each group of the divided group is either similar to the response of the pooled group or non significant. For instance, if the subdivided groups respond positive and negative to one of the land use categories, while the undivided group responds negatively, the set will not be discarded. In the second step, the categorisation with the highest number of significant p-values is chosen from the remaining set.

From the remaining set of FRGs, the one with the highest number of p-values below a threshold, in our case 0.05, is chosen. In case that several classifications are similar according to these criteria, the classification with smallest number of groups is preferred or if this is still not sufficient to rule out a single classification, the one with the lowest total sum of significant p-values is preferred. In case of multiple classifications are similar to these criteria, the one with the lowest number of positive significant responses is chosen. After calculating the optimised species groups and their response for each trait, we combine traits to get a final classification.

To test for plant group responsiveness, we designed a new null model which is related to the use of the 'lottery' model (Sale, 1978, Legendre *et al.*, 1997). A null model is a pattern generating procedure based on the randomisation of ecological data which generates new assemblage pattern that would be expected in the absence of the ecological mechanism of

interest (Gotelli & Graves, 1996). To design a null model it is therefore necessary to include every ecological pattern/mechanism except the one it is designed to test. Unlike the original 'lottery' model we use frequency data instead of presence/absence data. A species/frequency combination is drawn at random from a list of all observed species/frequencies regardless of the site. This combination is placed at a site until the site has reached the same total cover as recorded in the observation. In this way we keep the frequency distribution of each species similar to the frequency distribution in the observed data and can therefore account for the different size of species as well as for different colonising strategies e.g. clumped occurrence of tussock species.

Appendix 3. List of measured species and respective trait values or categories. Land use category: G – grazing, I – intermediate succession, A – advanced succession; Traits: SLA – Specific leaf area; LDMC – Leaf dry matter content; LNC – leaf nitrogen content; LCC – Leaf carbon content; LPC – Leaf phosphorus content; VH – Plant height; SM – Seed mass; LF – Life form: Th – therophytes, Geo – geophyte, hCr – hemicryptophyte, Ch – chamaephyte, nPh – nanophanerophyte; M – Type of mycorrhiza: NM – no mycorrhiza, AM – Arbuscular mycorrhiza, ECM - ectomycorrhiza; P – pollination mode: S- self-pollinated, W – wind, I – insect, M - mixed; D – Dispersal mode: U – unassisted, W – wind, E – exo-zoochory. n.a.. – not available. The values presented for continuous traits are the mean of several individual in each land use category.

Species name	Land use	SLA (m² kg⁻¹)	LDMC (mg g⁻¹)	LNC (mg g⁻¹)	LCC (mg g⁻¹)	LPC (mg g⁻¹)	VH (cm)	SM (mg)	LF	M	P	D
Agrostis pourretii	G	36.67	346.05	31.79	447.16	n.a.	16.85	0.02	Th	AM	W	E
Anthyllis lotoides	G	24.58	178.30	33.82	416.06	1.34	4.06	0.50	Th	AM	I	U
	I	22.64	194.44	31.28	406.09	1.18	5.94	0.71				
Biserrula pelecinus	G	19.34	234.57	29.07	465.13	1.39	5.50	0.43	Th	AM	I	E
Brachypodium distachyon	G	21.45	291.52	14.57	419.38	0.92	9.21	2.74	Th	AM	M	E
	I	30.38	303.44	13.30	439.41	0.69	15.37	2.35				
Carlina corymbosa	I	15.28	162.81	22.69	422.63	1.46	21.43	1.25	Geo	AM	M	W
Carlina racemosa	G	14.51	314.08	17.59	427.07	n.a.	16.43	0.79	Th	AM	I	W
Chamaemelum mixtum	G	20.78	171.97	24.60	423.33	1.73	13.33	0.11	Th	AM	I	U
Cistus ladanife	I	3.82	562.47	14.68	482.97	1.62	134.50	0.21	nPh	ECM	I	U
	A	3.89	554.70	17.28	483.93	1.36	139.27	0.19				
Genista hirsuta	I	19.29	358.99	38.39	472.49	1.24	73.90	2.56	nPh	AM	I	U
	A	24.22	368.57	32.76	468.43	1.12	80.23	2.47				
Helichrysum stoechas subsp. stoechas	I	13.96	290.96	13.69	463.39	1.31	42.38	0.02	Ch	AM	M	W
Holcus lanatus	G	28.58	250.23	15.41	429.01	1.59	15.12	0.08	hCr	AM	W	E

Appendix 3. (cont.) List of measured species and respective trait values or categories. Land use category: G – grazing, I – intermediate succession, A – advanced succession; Traits: SLA – Specific leaf area; LDMC – Leaf dry matter content; LNC – leaf nitrogen content; LCC – Leaf carbon content; LPC – Leaf phosphorus content; VH – Plant height; SM – Seed mass; LF – Life form: T – therophyte, G – geophyte, H – hemicryptophyte, C – chamaephyte, nPh – nanophanerophyte; M – Type of mycorrhiza: NM – no mycorrhiza, AM – Arbuscular mycorrhiza, ECM - ectomycorrhiza; P – pollination mode: S- self-pollinated, W – wind, I – insect, M - mixed; D – Dispersal mode: U – unassisted, W – wind, H – exo-zoochory, n.a.. – not available. The values presented for continuous traits are the mean of several individual in each land use category.

Species	Land use	SLA	LDMC	LNC	LCC	LPC	VH	SM	LF	M	P	D
Hypochoeris glabra	G	17.19	159.31	14.45	423.88	1.34	0.95	0.32	Th	AM	I	W
Lavandula stoechas subsp. luisieri	I	12.40	340.43	16.96	472.50	1.24	81.80	0.29	Ch	AM	I	U
Lavandula stoechas subsp. luisieri	A	16.46	342.02	16.53	470.81	1.19	69.50	0.29	Ch	AM	I	U
Lavandula viridis	I	12.91	298.94	16.93	479.78	1.48	56.70	1.59	Ch	AM	I	U
Lavandula viridis	A	13.86	271.44	19.75	476.28	n.a.	56.67	n.a.	Ch	AM	I	U
Lavandula viridis	G	23.76	158.34	29.54	410.88	1.53	2.38	0.22	Th	AM	I	U
Lavandula viridis	I	22.48	175.22	21.84	383.84	1.46	3.83	0.18	Th	AM	W	W
Logfia gallica	G	26.74	227.71	12.92	430.73	1.46	9.96	0.01	Th	AM	S	U
Ornithopus compressus	G	33.06	232.55	33.43	434.64	1.29	4.75	1.84	Th	AM	M	F.
Parentucellia viscosa	G	22.92	160.76	15.41	427.20	1.72	14.00	0.01	Th	AM	I	U
Parentucellia viscosa	I	25.51	176.71	26.35	443.50	1.74	14.80	0.01	Th	AM	I	U
Plantago coronopus	G	19.61	167.19	11.78	415.05	1.35	3.53	0.11	H	AM	W	U
Rumex acetosella subsp. angiocarpus	G	14.08	176.45	22.49	449.72	1.57	10.07	0.49	H	NM	W	U
Silene gallica	G	18.76	215.16	15.64	379.36	2.52	9.82	0.26	Th	NM	M	U
Tolpis barbata	G	27.09	128.25	21.20	405.05	1.63	11.39	0.11	Th	AM	M	W
Tolpis barbata	I	35.32	120.27	23.32	406.01	1.76	17.59	0.10	Th	AM	M	W
Trifolium angustifolium	G	16.71	281.97	28.85	449.57	1.19	9.28	1.08	Th	AM	I	W
Trifolium angustifolium	I	19.62	284.44	29.37	450.21	0.90	13.99	1.19	Th	AM	I	E

Appendix 3. (cont.) List of measured species and respective trait values or categories. Land use category: G – grazing, I – intermediate succession, A – advanced succession; Traits: SLA – Specific leaf area; LDMC – Leaf dry matter content; LNC – leaf nitrogen content; LCC – Leaf carbon content; LPC – Leaf phosphorus content; VH – Plant height; SM – Seed mass; LF – Life form: T – therophytes, G – geophyte, H – hemicryptophyte, C – chamaephyte, nPh – nanophanerophyte; M – Type of mycorrhiza: NM – no mycorrhiza, AM – Arbuscular mycorrhiza, ECM - ectomycorrhiza; P – pollination mode: S- self-pollinated, W – wind, I – insect, M - mixed; D – Dispersal mode: U – unassisted, W – wind, E – exo-zoochory. n.a.. – not available. The values presented for continuous traits are the mean of several individual in each land use category.

Species	LUC	SLA	LDMC	LNC	LCC	LPC	VH	SM	LF	M	P	D
Trifolium arvense	G	24.39	319.54	28.09	458.62	1.89	8.30	0.16	Th	AM	M	U
	I	23.07	243.67	27.54	467.36	1.01	12.07	0.21				
Trifolium bocconei	G	25.81	234.08	25.23	474.34	0.91	4.04	0.22	Th	AM	I	U
	I	23.34	303.60	24.88	468.68	0.94	5.36	0.14				
Trifolium campestre	G	41.29	236.43	38.15	423.93	1.34	4.02	0.15	Th	AM	M	U
	I	41.57	204.77	36.90	458.03	1.41	7.62	0.14				
Trifolium cherleri	G	24.40	265.30	25.17	446.46	0.75	2.19	1.43	Th	AM	I	U
Vulpia bromoides	G	22.82	373.16	8.26	435.34	1.09	13.22	0.17	Th	AM	S	E