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Dissertação de Mestrado
Mestrado em Ecologia e Gestão Ambiental

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Orientadores:

Professora Doutora Margarida Santos-Reis e Doutor Pedro Pinho

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Resumo

Uma vez que o consumo de carne e lactinícios tem vindo a aumentar no mundo, cada vez mais surge o desafio de desenvolver formas sustentáveis de produção. Assim, neste trabalho aborda-se também um aspecto essencial da alimentação humana, com uma forma de criação de gado sustentável, sem colocar em risco o futuro equilíbrio natural do ecossistema.

Os indicadores ecológicos têm vindo a ser crescentemente utilizados na monitorização de sistemas naturais, sendo definidos como características mensuráveis de estrutura, composição e função, que reflectem condições bióticas ou abióticas, processos e perturbações dos mesmos. O estudo e aplicação de indicadores ecológicos pode, portanto, tornar-se útil na averiguação de mudanças importantes nos ecossistemas.

De modo a combinar ambos os factores expressos anteriormente, ou seja, a produção de gado e a análise da mudança que esta pode causar, o objectivo do presente estudo foi selecionar indicadores ecológicos para o medir o impacto da pastorícia no ecossistema Montado.

Os Montados são sistemas agro-silvo-pastorais dinâmicos com múltiplas oportunidades de exploração humana, uma das quais a pastorícia. Para que essa exploração decorra da melhor forma, é necessário que o habitat se mantenha em boas condições ambientais. Há por isso uma forte relação de dependência entre a gestão que Homem faz e a sua estabilidade ecológica.

Para procurar seleccionar indicadores relevantes do efeito da pastorícia foram selecionadas duas áreas de estudo (Companhia das Lezírias (CL) e na Herdade da Ribeira Abaixo (HRA)) onde foram considerados dois tratamentos: pastoreio e exclusão de pastoreio (controlo). Na CL, o pastoreio refere-se a gado bovino e na HRA a ovino.

A exclusão ao pastoreio na CL ocorre desde 2008 enquanto a HRA não tem pastoreio nos locais de controlo há mais de 20 anos. Para cada tratamento, foram amostradas 9 áreas circulares de cerca de um hectare, totalizando trinta e seis sítios de amostragem.

A CL é uma propriedade do Estado, localizada em Samora Correia, a 40 km de Lisboa, com 18000 ha entregues à exploração agro-silvo-pastoril. Os solos da zona amostrada são maioritariamente arenosos. Em toda a extenção do espaço amostrado, não há grandes variações de inclinação do terreno. As suas zonas de pastoreio são Pastagens Permanentes Biodiversas Ricas em Leguminosas, que foram plantadas em 2007, no local amostrado, de forma a aumentar a produtividade pastoril e conteúdo em carbono da matéria orgânica do solo. Estas áreas de pastoreio são pastadas no meses de Outono e Inverno, período após o qual o gado bovino é transferido para outras pastagens.

A HRA está localizada na Serra de Grândola, em Grândola, 100 km a Sul de Lisboa. Neste estudo não só foi usada a área da HRA (221ha), para os locais de amostragem do tratamento sem pastoreio, como também propriedades circundantes, pastoreadas por gado ovino. Os seus solos são marcados pela presença de xistos e grauvaques, com litosolos pobres em matéria orgânica. Na HRA, a inclinação do terreno é distinta da CL, já que estamos numa zona de serra, com vários pequenos montes que surgem no espaço amostrado.

Os indicadores ecológicos seleccionados para avaliação incluíram líquenes epífitos, plantas vasculares, escaravelhos coprófagos e a caracterização elementar e isotópica de folhas de sobreiro. Os líquenes, plantas e escaravelhos coprófagos representam indicadores bióticos e a análise elementar e isotópica das folhas de sobreiro indicadores abióticos.

A amostragem decorreu durante os períodos de Janeiro e Fevereiro para os líquenes. Os líquenes foram amostrados na superfície do tronco dos sobreiros, seguindo o protocolo europeu standard. Entre os dois locais, 120 árvores foram amostradas no total. Foram registadas as espécies de macrolíquenes epifíticos e também a sua frequência. As espécies que não foi possível identificar no campo foram recolhidas e identificadas em laboratório. Os dados foram organizados por abundância de espécies e de grupos funcionais a que essas espécies pertencem ("oligotróficos", "mesotróficos" e "nitrófilos").

A amostragem de plantas decorreu em Maio de 2013. A frequência de plantas vasculares e cobertura de solo foram registadas segundo o método do ponto de intersecção, em que uma vareta metálica é colocada perpendicularmente ao solo e anotados os elementos que lhe toquem. Em cada sítio de amostragem foram efetuados dois transectos de 10m, com 21 pontos regularmente espaçado por transeto. Os indicadores amostrados foram os grupos funcionais quanto à forma de crescimento (arbustivas, basais e semi-basais, erectas, em tufos e trepadeiras. Os indicadores do solo foram solo nú, líquenes, musgo, pedras, folhada, e vegetação morta.

Os insectos coprófagos foram amostrados através de duas armadilhas de queda em cada sítio de amostragem. Uma delas foi colocada em espaço aberto (sem cobertura de vegetação), e a outra em espaço fechado (coberta por árvores e/ou arbustos). Cada armadilha consistia num pequeno recipiente, coberto por uma rede plástica sobre a qual foram colocados excrementos de vaca. Foram deixadas no local durante 48 horas. Os insectos recolhidos foram trazidos para laboratório para triagem, tendo sido isolados os escaravelhos coprófagos da subfamília *Aphodiinae* e das famílias *Scarabaeidae* e *Geotrupidae*, e esses foram identificados ao nível da espécie. Os dados foram organizados por abundância de espécies e por grupos funcionais a que essas espécies pertencem ("tunnelers" e "dwellers"), sendo divididos também por tamanho (pequenos e grandes).

As folhas de sobreiro foram recolhidas das mesmas árvores onde os líquenes foram amostrados, também em Janeiro e Fevereiro de 2013. Cerca de 10 folhas de cada árvore foram secas, moídas e analisadas quando ao seu conteúdo em Carbono e Azoto e respectivos isótopos. Os indicadores resultantes foram o rácio Carbono/Azoto, o $\delta 13C$ e o $\delta 15N$.

O processo de tratamento estatístico dos dados envolveu análises multivariadas (DCCA/RDA/CCA) e univariada não-paramétrica (testes U de Mann-Whitney). Começou por realizar-se uma análise multivariada das espécies de líquenes e insetos coprófagos, para compreender a composição das comunidades e a sua relação com o pastoreio. Os resultados mostraram que o principal factor organizativo das comunidades não foi o pastoreio, mas sim as áreas de estudo e/ou a espécie de gado. Prosseguiu-se testando os restantes indicadores baseados em grupos funcionais e na análise elementar e isotópica das folhas. Estes indicadores foram comparados considerando o tratamento e a área de estudo, através de testes não paramétricos U de Mann-Whitney. e graficamente representados em boxplots. De acordo com os resultados, os indicadores foram distribuídos por três grupos. O primeiro contém os que são significativamente diferentes para o efeito do tratamento nas duas áreas de estudo. O segundo grupo, contém os indicadores que só são estatisticamente distintos no tratamento numa das áreas de estudo. O terceiro, tem aqueles que, ou mostram tendências opostas nas duas áreas de estudo, ou não têm diferenças com significância estatítica em nenhuma das duas. Integraram o primeiro grupo os arbustos, as plantas semi-basais, e escaravelhos coprófagos “tunnelers” (escavadores de túneis) de tamanho pequeno. Estes três indicadores foram assim apontados como melhores indicadores para o impacto da pastorícia no Montado.

De seguida foi feita uma análise multivariada com os indicadores do primeiro grupo. O passo seguinte foi incluir outras variáveis ambientais através de uma análise parcial. Deste modo, averiguou-se o efeito que estas novas variáveis (usadas como covariáveis) terão nos dados iniciais, procurando-se isolar o efeito da pastorícia. Esta análise permitiu verificar que as variáveis radiação solar potencial, inclinação e densidade de árvores em cada local de amostragem não tiveram influência no resultado obtido anteriormente.

Finalmente, através dos "scores" do primeiro eixo da análise parcial foi construído um índice de integridade ecológica que ajudou a entender de que forma os espaços são impactados pela pastorícia.

O índice resultante apontou a seguinte ordenação das áreas de estudo e tratamentos: em primeiro lugar, como menos impactada surgiu a HRA não pastoreada. Segui-se a CL também não pastoreada. Em terceiro, ficou a CL pastoreada, e por último, ou seja, a mais impactada pela actividade pastoril, a HRA pastoreada.

Concluindo, esta ordenação permite compreender melhor o impacto da pastorícia nas áreas estudadas. Os usos deste índice podem estender-se a outras problemáticas ambientais (tal como o abandono agrícola e as actividades de exploração florestal), e são uma boa ferramenta de análise e de informação para o público geral ou para gestores e políticos. Adicionalmente, poderão ajudar na monitorização de impactos da actividade humana e do estado do ecossistema em locais de relevância ecológica, na avaliação de acções de conservação e na prioritização alvos de novas medidas de protecção ambiental.

Palavras-chave: pastoreio; Montado; multi-taxa; indicadores ecológicos; gestão ambiental

Context

This project is focused in studying the application of ecological indicators to assess the impacts of human activities. One of the activities that is essential to humans and also has an impact on ecosystems is grazing. It was approached as the main problem in this project because of how vital it is to feed humankind and to provide a balanced economic exploitation of the land. The *Montado* is an ecosystem where grazing plays a very important role. In few other places there is such equilibrium between human intervention, ecological preservation and biological diversity.

If not managed properly, grazing is an activity that can become self-limiting. When the regime is too intensive, trampling and vegetation consumption exhaust the capacity of the habitat to sustain pastures, ending the possibility of further exploration. However, with the absence of grazing, there is an increase in shrub density that can alter the vegetation structure, as the system moves to another phase of the ecological succession. This may transform the landscape in a manner that the *Montado* can no longer be recognized as one, evolving to a shrubland. Once this happens, human exploitation of the land becomes increasingly hard.

From the beginning, the selection of the different taxonomical groups that would take part in the project was a straight-forward process. Initially, there was a very wide range of possibilities. The indicators that were integrated in this study fulfilled the conditions of both being quickly and easily put in practice during the course of just one year. The input from specialists in different areas was very helpful in confirming their adequacy for the problem at hand. As in Nature a lot of interactions that regulate the ecosystems are interconnected as a complex network, a single indicator may not be enough to capture all this variability. Therefore, this study focuses in the combination of several of them, to strengthen their potential as a whole. The number of studies with multiple taxa, however, is rather low, reinforcing the need for a new one.

Moreover, another consideration to be taken into account and that helped in selecting the main problematic hereby studied is the human perspective. More than considering what the impacts on the environment alone are, there should be a specific concern with the economical side of the management of the sites. These sites are being exploited for profit that guarantees the livelihood of some people, through the diverse services they can offer. It is then useful to understand how that exploitation may undermine itself, in order to protect the future of the land, from an ecological and economic standpoint. By ensuring these two components are kept on the table, it becomes easier to manage a healthy ecosystem.

To land managers in *Montado* areas, it is particularly relevant to know and understand which the best procedures are, especially at the long-term, in order to preserve the space that provides their livelihood. One of the strong points of this study, and others alike, is to provide managers with the necessary tools to make the best decisions, while being responsible towards biodiversity and the environment. On the other hand, in most cases it is hard for them to access the information and conclusions that the scientific community provides. Academia should commit to making an effort to disseminate the benefits of their work and make them more readily available.

For being a very sensitive habitat and for its role on the conservation of biodiversity, being a biodiversity hotspot, the *Montado* deserves special attention. Other factor that makes it so unique is the number of possible economical activities in the same space and the opportunities it provides in helping local communities in a healthy and environmentally friendly way. Although they are not discussed in this work, other goods and services provided by *Montado* include mushrooms, medicinal herbs and timber, the aesthetical value of the land, its ability to provide leisure activities and job creation in rural areas.

In Portugal, ecological indicators are not widely applied yet. This work intends to contribute to foster its application. The timing for this approach seems the right one since society – not only in Portugal, but worldwide - is increasingly worried about environmental issues, wanting to be better informed. On the other hand, the economic crisis asks for more focused investments. Indicators reduce the cost and time investment in ecosystems monitoring and public information. For being about efficiency and effectiveness and for many other reasons, this area of research bares strong opportunities for the future. Also due to the current economic crisis, some people are returning to the rural areas, which may increase agricultural activity in a near future. At the moment, developing monitoring tools to accompany the additional impacts of change in land use seems to be particularly relevant.

New research projects, and resulting papers, increase in number every year making it increasingly hard to have fresh ideas. The current project took a path that was not found in the literature. While it applies many of the concepts in use in other works, the approach to the data is conducted in an innovative way, combining the previous knowledge with a new perspective.

The context of the *Montado* including: its sensitivity, ecological and economic importance, academia interaction with managers, its relevance for Portugal and other countries and the role that grazing may play on the ecosystem has been put forth. Now, it is time to advance with the application of ecological indicators for studying the effects of grazing.

Article

Title: Ecological indicators of grazing effects in cork oak woodlands: an integrated approach

1. Abstract

Lichens, vascular plants, coprophagous beetles and cork oak leaves were assessed as to their effectiveness as ecological indicators for the effects of grazing in cork oak woodlands (*Montados*). More than considering the individual information gathered from each of these groups, our goal was to integrate them, in order to simplify the complex interactions established among them. The sampling was performed in two study areas, one grazed by cows and the other by sheep, and two treatments were considered at each: grazed and grazing-excluded (control). Indicators based on species (community organization), functional groups and abiotic factors were tested. The results showed that communities' organization of lichens and coprophagous beetles was more related to the study area/livestock species rather than to the grazing effect. However, some of the functional groups proved to be robust indicators for the effects of grazing. On the basis of such information an integrative Index of Ecological Integrity was built, using the best individual indicators that assisted us in comparing the relative magnitude of grazing impact on the study areas. This index can be tested at a broader scale, involving other pastoral systems, and also be used by land managers and policy-makers to prioritize measures of conservation or protection of the environment.

Highlights

- This study looks for indicators for the effect of grazing in the *Montado* ecosystem.
- Lichens, plants, coprophagous beetles and cork oak leaves were tested.
- Plants and coprophagous beetles responded more clearly to grazing.
- An integrative Index of Ecological Integrity was built to compare the study areas.

Keywords: ecological integrity index; multi-taxa; *Montado* ecosystem; land management.

2. Introduction

An indicator provides evidence for trends or phenomena that are not immediately detectable (Hammond et al., 1995). Environmental indicators are often interchangeable with the term of ecological indicators (Niemi and McDonald, 2004), although ecological indicators should be considered a subset of environmental indicators (NRC, 2000) that tries to narrow down from the complexity of all the elements in a causal chain that links human activities to environmental and social responses to some impacts (Smeets and Weterings, 1999). Environmental indicators then focus on isolating the key aspects of the environmental condition, large-scale patterns and appropriate actions (Niemeijer, 2002).

Niemi and McDonald (2004) combine the terms by Young and Sanzone (2002) (USEPA) and the Hierarchy of Noss (1990) to define ecological indicators as: "measurable characteristics of the structure (e.g., genetic, population, habitat, and landscape pattern), composition (e.g., genes, species, populations, communities, and landscape types), or function (e.g., genetic, demographic/life history, ecosystem, and landscape disturbance processes) of ecological systems”. These indicators can reflect biotic condition, chemical and physical characteristics, ecological processes, and disturbance (Harwell et al., 1999; Young and Sanzone, 2002). They are frequently biological in nature, due to their sensitive response to chemical, physical and other biological phenomena (Niemi and McDonald, 2004). The most common roles they assume are to become an early-warning system (through the assessment of the state of the environment) or to go to the root cause of disturbance (Dale and Beyeler, 2001). In the present work, several variables are going to be evaluated as ecological indicators on their effectiveness in detecting the impacts and changes on the environment that originate from grazing activity.

Livestock management is an increasingly hot topic in the twenty-first century, as the population and meat and dairy consumption increase (FAO, 2002). Integrating the animals in a healthy environment for their growth and preserving the pastures and ecosystems they feed on can be a challenge and a research priority. Here, the two livestock species being studied are cattle and sheep. Among the main impacts these animals generate with their feeding habits and roaming behaviour are vegetation reduction, trampling, and dung and urine production (Perevolotsky and Seligman, 1999). However, grazing also has its benefits such as reducing the need for mechanic vegetation clearing, which costs money and can disturb the soil balance. Livestock also is responsible for slowing down succession, impeding the evolution of grasslands to shrublands and then forests. Land managers are interested in preserving the balance of the Montado as it is, because that state allows them to better exploit the land, and to continue their activity, without an increase in biomass becoming an obstacle, through the overdevelopment of shrubs (Chapin III et al., 2002). Non-grazing retards the transition of pastures to shrubs and trees and shrub association (Bernáldez, 1991; Sluiter and de Jong, 2007; Tasser et al., 2007; Tzanopoulos et al., 2007; Verburg et al., 2009). The extent to which this change impacts the *Montado* ecosystem will be studied in the present work.

The *Montado* is an agro-sylvo-pastoral system, shaped and explored by humans, in a way that has produced a co-dependence relationship between human activities and the forest to foster its sustainability. This system has a savannah-like physiognomy, and in Portugal is dominated by two oak species: cork (*Quercus suber*) and holm (*Quercus ilex subsp. ballota*), with varying densities (Pinto-Correia et al., 2011). It is concentrated mainly in the Alentejo region, where 730.000 of the total 800.000 ha in the country are located. In Spain, the equivalent system is named *dehesa.* Spain has an area of about 2 million hectares of this ecosystem (Pinto-Correia et al., 2011). Besides the Iberian Peninsula, these woodlands can be found scattered around the western-Mediterranean, in both the south of Europe and North Africa. Its geographical distribution (region considered a biodiversity hotspot – Myers et al., 2000), economic significance and natural value demand strong research contributing to its long-term sustainability.

Grazing is a functional layer of the system and is instrumental for its preservation. The *Montado* system, due to its complexity resulting from multiple activities, requires control and science-based advice in order to maintain an equilibrium and subsequent sustainability (Pinheiro et al., 2008; Pinto-Correia et al., 2011; Ribeiro et al., 2004). It is this equilibrium that ensures profitability, in a sector that is so important to the Portuguese economy. The desire for the maintenance of this stability becomes more understandable when we consider the fact that Portugal has 33% of the area of cork oak in the world and is responsible for 54% of the world cork production (Ribeiro et al. 2010).

The concept of ecological integrity (also known as ecosystem health) is becoming more relevant in ecological research, although not without some controversy. Some authors relate it to human health, while others insist on how it deserves its own definition. Generally, an ecosystem can be said to be healthy if it retains its stability and sustainability, while maintaining its organization and being resilient to change and impact as it provides for human needs (Haskell et al., 1992; Woodley et al., 1993; Sampson et al., 1994; Vora, 1997). The concept seems to be perfectly aligned with the objectives of this study. The *Montado* is one of the systems where the ecological balance is most at stake, and where the need to keep providing goods and services is essential for both ends (ecosystem and humans). Ecological integrity is a dynamic concept, involving structure, function and composition of the environment, resilience and stability. Many variations of these components are combined into definitions proposed by Cairns (1997), Shackell and colleagues (1993), among others. These definitions revolve around the same conceptual core, being similar and using the properties that are listed above. The main problem with a concept like this is perhaps the definition of the equilibrium state. In cases other than semi-natural ecosystems, it can be feasible to find a more "pristine, natural" site – and even that is becoming hard, now that humans influence the ecosystems everywhere on Earth in such a tremendous way (Zalasiewicz et al., 2010). The word health itself offers an aid to interpret integrity as lack of "disease", meaning no symptoms or signs of degradation and decaying quality or performance. This metaphor was proposed by Schaeffer and others (1988), and Rapport (1989, 1992, 1995a,b), to easily convey the message of how ecosystems function to the general public. For being (1) sufficiently sensitive to provide an early warning of change; (2) distributed over a broad geographical area, or otherwise widely applicable; (3) capable of providing a continuous assessment over a wide range of stress; (4) relatively independent of sample size; (5) easy and cost-effective to measure, collect, assay, and/or calculate; (6) able to differentiate between natural cycles or trends and those induced by anthropogenic stress; and (7) relevant to ecologically significant phenomena (Cook, 1976; Sheehan, 1984; Munn, 1988; Noss, 1990), good indicators are intended to be the best tool to capture those "symptoms" in the most efficient way. The diagnosis is the only way to prevent "death" when it is still safe to correct practices. The combination of sensitivity, traceable change over time and ease of data gathering constitute unique advantages to make this diagnosis worth-while to keep doing regularly.

In this work four biological groups will be tested as ecological indicators: lichens, vascular plants, coprophagous beetles and cork oak leaves. These are groups whose methods and application was successful in previous examples (Herrick et al., 2009; Pinho et al., 2011; Hortal and Lobo, 2005; Bai et al., 2012). Bearing in mind the biological groups this study goes over, it could be added that they reflect different stages of the nitrogen cycle (Dawson et al., 2002; Garnier et al., 2007; Nichols et al., 2008; Pinho et al., 2011). However, an integration of these groups may provide an innovative approach and be more efficient by retaining the complexity of interactions between the system components. Other than being known to work in isolation, the reasons why these indicators were selected were the knowledge on the ecology of these groups and the quickness and low difficulty of application, making their use for grazing possible.

Knowing the negative impacts that grazing may have on the system of *Montado*, the question is if those impacts are big enough to make grazing very influential on the habitat dynamics. If the answer is positive, then this can mean the same indicators that are able to reflect grazing impacts, may also be adequate for ecosystem health, in a broader way. Bearing in mind the biological groups this study goes over, it could also be added that they reflect different stages of the nitrogen cycle (Dawson et al., 2002; Garnier et al., 2007; Nichols et al., 2008; Pinho et al., 2011).

A meta-analysis of the current state of research in this field was performed. In Google Scholar, out of 30.400 results for “ecological indicators”, only 109 also included the word “multi-taxa”, a very low ratio of 0.36%, and 54 of those 109 were performed since 2009, demonstrating that this trend is recent. If the word “Mediterranean” is included, the number drops down to 35 studies; only 29 for “grazing”, “ecological indicators” and “multi-taxa”. These numbers highlight the innovation of this study and the need for this and more studies alike in the future.

The main objective of the work was to select indicators for the analysis of the impacts of grazing. This was carried out on two different study areas, which are grazed by two different livestock species. The results are expected to provide an evaluation of current state and future tendencies of the ecosystem integrity and also tools for human management of *Montados*. The ever-expanding science of biological indicators is being increasingly applied and becoming a part of bigger organizations, responsible for large-scale operations. To know the extent to which this evolution is taking place, an overlook of the application of indicators on different scales will also be presented in this paper.

3. Methods

3.1 Study areas

This work was conducted in Portugal, south-west Europe, in two areas with different geomorphologic and pasture characteristics (Appendix I): Companhia das Lezírias (CL) and Herdade da Ribeira Abaixo (HRA).

Both study areas are characterized by a Mediterranean climate and are mainly covered by savannah-like cork oak (*Q. suber*) woodlands. The two areas are Long Term Ecological Research sites (LTER) devoted to the study of these woodlands and are under an extensive grazing regime (cows at CL and sheep at HRA) that includes low livestock density and a rotation system.

CL is located c. 40 km East of Lisbon, in the municipality of Samora Correia (38º 52' N, 08º 51' W). It is a state-owned property (18000 ha) devoted to agro-sylvo-pastoral activities. The mean annual temperature is 16.3 ºC and annual rainfall averages 700 mm (local weather station, 2002 to 2010). CL is located in a plain area with sandy soils that were sowed with biodiverse pastures in 2007, on the basis of a legume-rich seed mixture, aiming to increase pasture productivity and the carbon content of the soil organic matter (Teixeira et al. 2011). Sowing was done in 2007 and the areas were thereafter pastured. On the sampling year, there was grazing by cattle on two periods: one from 25/09/12 until 17/12/12 (83 days) and another between 25/01/13 and 12/04/13 (72 days). The stocking rate was 1.24 LSU (livestock units) /ha.

HRA is located in Serra de Grândola, in the municipality of Grândola (38º 05’ N - 38º 08’ N; 8º 33’ W - 8º 38’ W), 100 km south of Lisbon. Besides this state-owned property (221ha), mostly used for scientific research (Field Station of the Centre for Environmental Biology), this study area further includes areas from the adjoining private owned proprieties, totalizing an extra 61 ha. The mean annual temperature is 15.6ºC and the annual rainfall averages 500 mm (Comissão Nacional do Ambiente 1983; 1967 to 1980). HRA is located in a moderate rolling mountain (Serra de Grândola) dominated by schist and greywacke rocks that are covered with lithosols poor in organic matter (Pereira et al. 2009). In this area, pastures are natural and have been continuously grazed by sheep (c. 0.76-0.95 LSU/ha).

3.2 Sampling design

To establish ecological indicators for the effects of grazing two treatments were compared in each study area (CL and HRA): grazing and grazing-exclusion (control). In CL grazing excluded sites (control) were fenced in 2008, while at HRA these have been devoid of grazing for more than 20 years. For each treatment, in areas with homogeneous tree cover, nine 1 ha circles, were sampled for biotic (lichens, vascular plants, and coprophagous beetles species richness and functional groups) and abiotic (tree leaves elemental and isotopic composition) parameters, totalising 36 sampling sites (2 areas*2 treatments*9 replicates).

3.2.1 Lichens

During January and February 2013, the 3-4 mature cork oaks closest to the centroid of each sampling site were surveyed for epiphytic macrolichen diversity (total of 120 trees). The sampling procedure was one adaption of the standard European protocol (Asta et al., 2002), already tested in Mediterranean ecosystems (e.g. Stofer et al. 2006, Pinho et al. 2011,2012). Sampled trees had a perimeter >65 cm, nearly straight trunks (< 10º inclination from a vertical position) and a healthy appearance. Sampling was done always in virgin cork, i.e., either on trees which were never harvested or above the level of cork extraction. On each tree trunk, a vertical sampling grid (five 10cm x 10cm squares) was placed 130 to 210 cm from the ground at each of the four main cardinal orientations (N, S, W, E). Observed species were then recorded as present in a frequency of 1 to 5, depending on the number of grid squares they were identified on.

We additionally recorded GPS coordinates, tree perimeter at breast height, and sampling height. Most species were identified in the field but when needed samples were collected for laboratorial identification. Small species of the *Usnea* genus posed identification problems and were recorded as *Usnea spp*, with the exception of *Usnea rubicunda,* an easier species to identify. Nomenclature used follows the online database ITALIC (Nimis and Martellos, 2008). Species richness and frequency of functional groups regarding nitrogen tolerance (Nimis and Martellos 2008) were calculated.

3.2.2 Vascular plants & soil cover

During May 2013, vascular plants & soil cover were sampled using the point-intercept method, adapted from (Herrick et al., 2009). For each sampling site, two 10 m transects (72 in total) with 0.5 m separated points (21 points/transect) were used. At each point all plants touching the sampling pole (a thin metal pole positioned orthogonally to the ground) were recorded according to growth form (functional groups): shrubs, rosettes, semi-rosettes, erect leafy, tussock forming and climbers. Whenever necessary, samples were collected for clarification in the laboratory. Soil cover categories were: bare soil, lichens, mosses, rocks, dead leaves and plant litter. All transects were georeferenced. Data were expressed in occurrence frequency.

3.2.3. Coprophagous beetles

During May 2013, following Hortal and Lobo (2005) approach, a pair of pitfall traps were placed at each sampling site, one in an open area (exposed to sunlight) and another in a closed one (shaded by trees or shrubs) (total of 72 traps). The distance between traps was no less than 20 m. Each pitfall consisted of a large plastic container (diameter: 26 cm; depth: 10 cm) buried and levelled to the ground, topped by a rigid plastic net with about 250 g of bait (cattle dung) placed over it. Following the recommendations by Lobo and colleagues (1988) and Veiga and others (1989), the baited traps were set in place for 48h. All collected insects were preserved in 70% alcohol and brought to the laboratory for identification. In the laboratory, the coprophagous beetles of the subfamilies *Aphodiinae*, *Scarabaeidae*, and family *Geotrupidae* (superfamily *Scarabaeoidea*) were separated from other by-catch organisms and identified to the species-level using Baraud (1992). Veiga (1998) and Dellacasa and Dellacasa (2006) were used for *Aphodiinae* and Piera and Colón (2000) for *Scarabaeidae* and *Geotrupidae*. Coprophagous beetles' richness and frequency of functional groups regarding size (small/large tunnelers and dwellers) were calculated.

3.2.4 Tree leaves

During January and February 2013 a composite sample of leaves was taken on each sampling site, considering 10 fully expanded leaves collected from sunlit parts of the canopy (3-4 leaves from each tree sampled for lichens). The leaves were then ground to a fine powder using a ball mill (MM 2000, Glen Creston) after being dried for 72 hours (temperature 60 ºC). 13C/12C and 15N/14N ratios in the samples were determined by continuous flow isotope mass spectrometry (CF-IRMS) (Preston and Owens, 1983), on a Hydra 20-22 (Sercon, UK) stable isotope ratio mass spectrometer, coupled to an EuroEA (EuroVector, Italy) elemental analyser for online sample preparation by Dumas-combustion. The standards used were IAEA-N1 and USGS-35 for nitrogen isotope ratio, and IAEA-CH6 and IAEA-CH7 for carbon isotope ratio; δ15N results were referred to Air and δ13C to PeeDee Belemnite (PDB). Precision of the isotope ratio analysis, calculated using values from 6 to 9 replicates of laboratory standard material interspersed among samples in every batch analysis, was ≤ 0.2‰. Elemental composition on N and C (%N and %C) and isotopic composition (δ15N, δ13C) were determined by the Stable Isotopes and Instrumental Analysis Facility (SIIAF) of the Centre for Environmental Biology (CBA), University of Lisbon - Portugal.

3.3. Data analysis

We used a two-step analytical approach. First, we tested which variables could be used as ecological indicators of grazing effects and then used those variables to build an Index of Ecological Integrity.

Two types of biotic variables (communities' composition - lichens or coprophagous beetles; functional groups - lichens, vascular plants and coprophagous beetles) and one abiotic (isotopic composition of tree leaves) were tested as ecological indicators.

3.3.1. Selection of variables as indicators for the effect of grazing

Communities' composition was tested through direct ordination analysis, after determining if relationships were linear or unimodal. This was done using Detrended Canonical Correspondence Analysis (DCCA) which measures the beta diversity in community composition along species-dependent gradients (i.e., not accounting for environmental variables). According to Leps and Smilauer (2003) the reported "gradient length" was used to choose either a linear (RDA, Redundancy Analysis, for gradient lengths smaller than 4) or an unimodal ordination (CCA, Canonical Correspondence Analysis, for gradient lengths larger than 4). In both approaches, site and treatment were used as environmental variables. If the main communities' axis was governed by the grazing/grazing exclusion treatment the communities' composition was kept for further analysis, being discarded if the study area emerged as the main factor. Results were represented as scatterplots of variables and environmental variables.

Functional groups and leaf isotopic composition were tested looking for significant differences between treatment and study areas. As variables' distribution approached but not fulfilled normality, the non-parametric Mann-Whitney's U rank-test was performed to assess the statistical significance of observed differences. Variables presenting significant differences between treatments and/or study areas were chosen for subsequent analysis. Results were represented as boxplots.

3.3.2 Index of Ecological Integrity

To build the Index of Ecological Integrity, a direct ordination analysis was carried out on the selected variables, considering treatment and study areas as environmental variables. As weighting factors of each variable, we used the respective scores obtained on the first axis, which were multiplied by the standardized values of each variable. The index was calculated for each sampling site, and for comparison between study areas we used boxplots.

An additional test was carried out to assess the potential influence of other environmental factors on this index. This was done by performing a partial RDA with the slope, annual potential solar radiation and tree density as covariables. These variables were chosen from a set of abiotic variables that more likely could influence the tested ecological indicators. Slope and annual potential solar radiation were calculated in ArcGis 10 (ESRI 2010) using a digital terrain model with 10 m resolution, from national cartography (1:25000). Potential Solar Radiation measures the amount of energy potentially arriving to each sampling site, and considers both the site and the nearby orography. Tree density was determined within each sampling site by manual interpretation of aerial orthophoto maps. The data was stored and organized for statistical analysis in an excel datasheet file (Microsoft Excel, 2010). The boxplots and Mann-Whitney's U tests were performed using STATISTICA software (Statsoft Inc., 2010) and all multivariate analyses were run using the software package CANOCO (ver. 4.5, ter Braak and Smilauer 2003).

4. Results

4.1 Communities analysis

A total of 22 lichen species was identified in this study. Species DCCA analysis resulted in a maximal gradient length of 4.980, indicating the use of the unimodal ordination method (CCA). The plot of the two first axis of the CCA (accounting for 44.5% and 12.9% variance respectively) revealed that communities were grouped according to location rather than by treatment (Fig. 4.1). Species more associated to study area CL, such as *Physconia grisea*, *Ramalina fastigiata*, *Parmotrema hypoleucinum*, *Usnea rubicunda*, were located in the positive side of the first axis. Species more associated to HRA, such as *Parmotrema sulcata*, *Parmotrema reticulatum*, *Parmotrema arnoldii*, were located in the left negative side of the first axis.

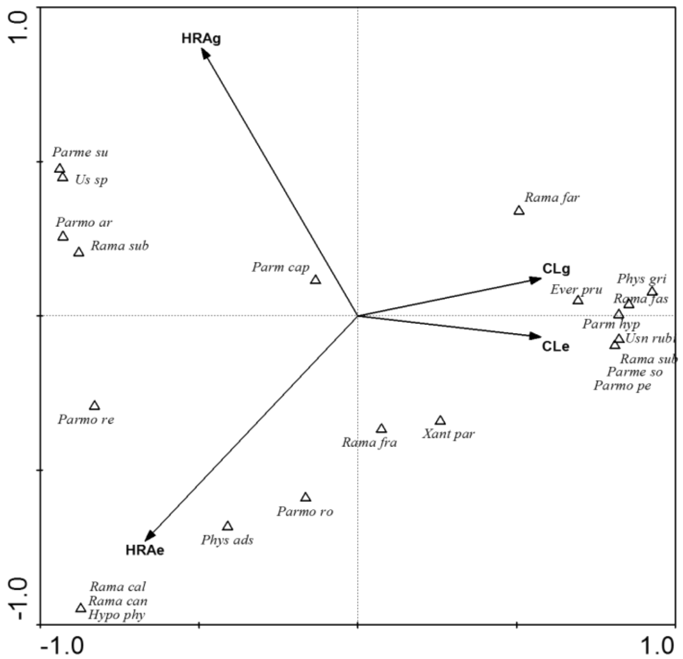


Fig. 4.1 – Ordination diagram (CCA) with all lichen species and the study areas separated by treatment as environmental variables. "e" is for grazing excluded, "g" for grazed. 44.5% and 12.9% variance was explained by the first and second axis, respectively.

A total of 14 coprophagous beetles species (each with more than 20 captured individuals) were used to perform the DCCA analysis, resulting in a maximal gradient length of 1.683. The two first axis of the linear ordination analysis (RDA) accounted for 47.3% and 3.1% of the species variance (Fig. 4.2). Again, communities were found to be organized by study area rather than treatment. Traps placed in open and closed vegetation belonging to the same study area and treatment were located close in the plot, with CL grazing-excluded sampling sites being further apart. Interestingly, most species were located on the negative portion of the second axis, together with the grazing treatments, suggesting that a higher frequency of most species was associated with grazing for both study areas.

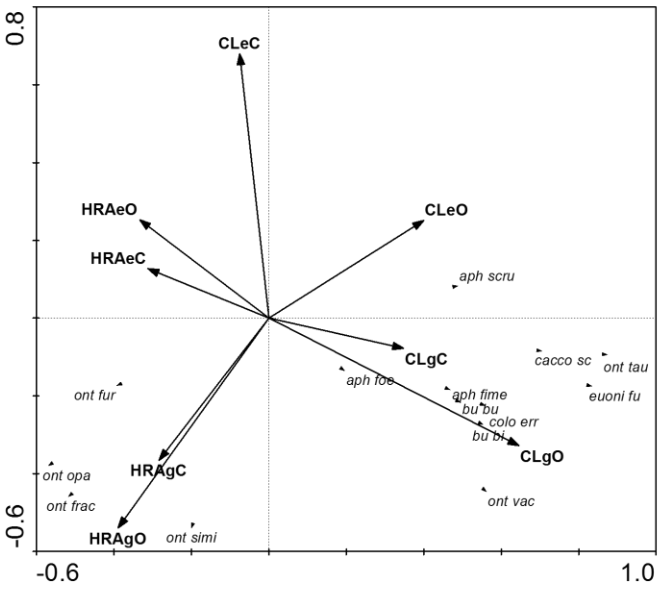


Fig. 4.2 - Ordination diagram (RDA) with the coprophagous beetles species having a representation of more than 20 captured individuals, divided by (C)losed and (O)pen setting, and the study areas separated by treatment as environmental variables. "e" is for grazing excluded, "g" for grazed. 47.3% and 3.1% variance was explained by the first and second axis, respectively. (The lines of the arrows of the species were removed for graphical clarity)

Lichens and coprophagous beetle species were re-analysed together. The preceding DCCA resulted in a maximal gradient length of 2.025, pointing to the linear ordination method. The results of the consequent RDA accounted for 59.0% and 6.1% of the species variance (see Appendix II). As with previous analysis, communities were grouped by study area, rather than treatment.

4.2 Functional groups and isotopic composition

Functional groups of lichens, plants, coprophagous beetles and leaves' isotopic composition were individually tested as ecological indicators of the effects of grazing. According to the results of the Mann-Whitney's U tests, three groups of indicators were separated. The first group includes the indicators which allow the distinction between the treatments for both study areas. More specifically, to be in this group, the indicators must reveal a significant difference between grazing and grazing-exclusion plots in both study areas, and the direction of the difference must be the same (i.e., either increasing or decreasing in both study areas). The second group includes indicators that have significant differences between treatments only in one study area, while the direction of the trend is the same in the two areas. Finally, a third group included indicators that result in non-significant differences between treatments or result in contrasting trends for the study areas.

The frequency of semi-rosettes, shrubs and number of small tunnelers showed significant differences between treatments for both study areas (p-values in Table 1, below) (Fig. 4.3). Shrubs were almost non-existing in CLg, and have a low abundance in HRAg. In both study areas, there is a significant increase of shrubs in the respective grazing excluded sampling sites. Semi-Rosettes showed the oposite trend, presenting a low frequency on the grazing-exclusion sampling sites and increasing in the grazed ones. The number of small tunnelers per trap presented the same trend as semi-rosettes, being significantly higher in the grazed sampling sites.

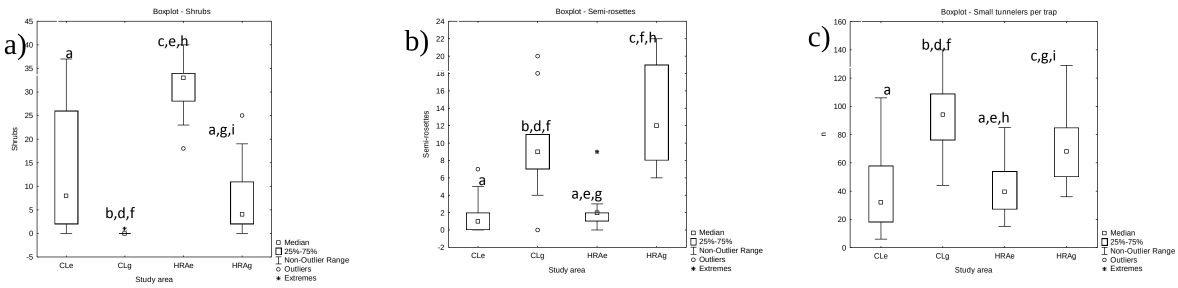


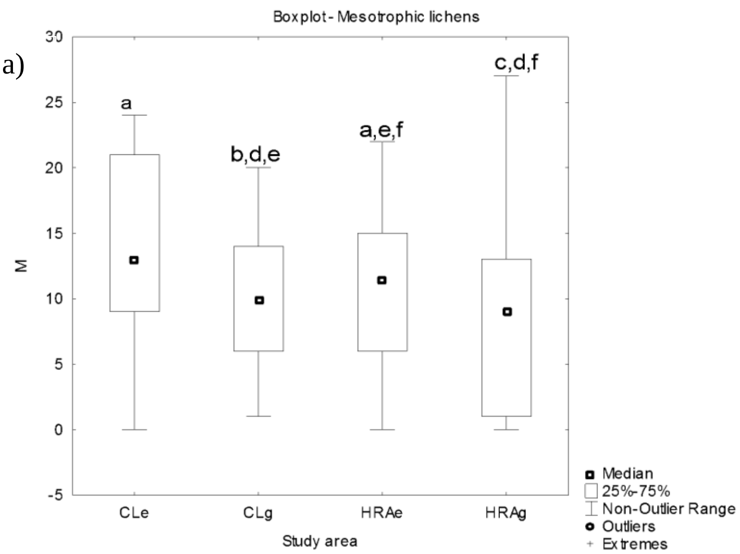
Fig. 4.3 - Boxplots for thefrequency of shrubs (a), semi-rosettes (b) and number of small tunnelers per trap (c). Different letters indicate statistically significant difference (Mann-Whitney's U test; p values in Table 1). "e" is for grazing excluded, "g" for grazed.

Table 1 – p-values for the Mann-Whitney's U test performed for the boxplots in Fig. 4.3.

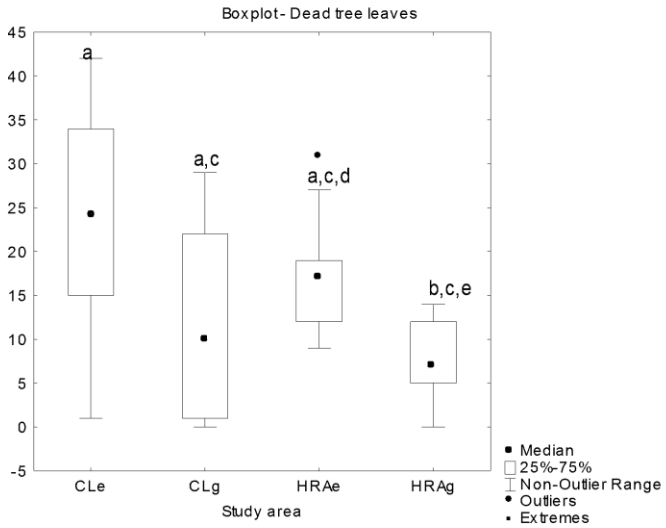
	CLe/CLg	CLe/HRAe	CLe/HRAg	CLg/HRAe	CLg/HRAg	HRAe/HRAg
Bushes	**0,002316**	**0,027276**	0,452913	**0,000412**	**0,008072**	**0,001086**
Semi-Rosettes	**0.008072**	0,723932	**0,000792**	**0,011849**	0,289316	**0,001086**
Small Tunnelers	**0,000156**	0,669291	**0,005366**	**0,000012**	**0,027887**	**0,000597**

The second group comprised the majority of the indicators tested (see Fig. 4.4). Mesotrophic lichens decreased slightly but not significantly with grazing in HRA, but significantly in CL. Most vascular plants and soil cover indicators were also found in this group: frequency of dead leaves, rocks, bare soil, erect leafy. Coprophagous beetles were represented though big tunnelers and small dwellers, with both showing significative differences in CL, but not in HRA. Tree leaves carbon/nitrogen ratio and δ15N also showed a statistical trend but only for one study area (HRA).

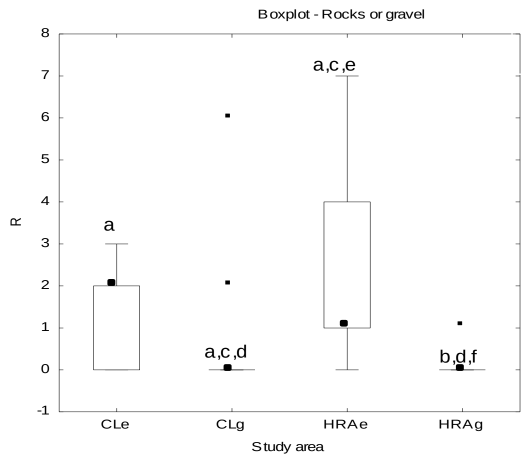
a)



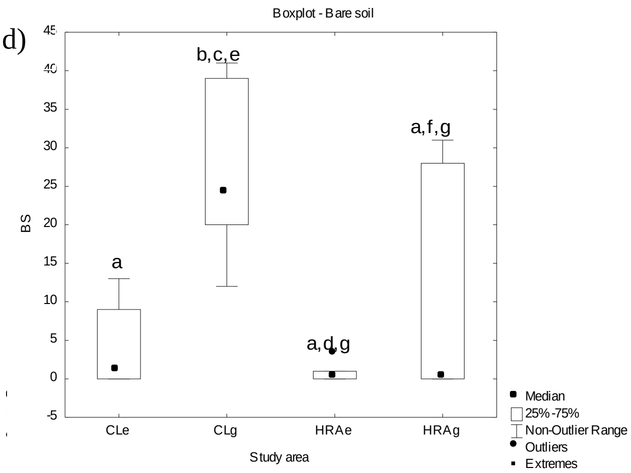
b)


Boxplot - Dead tree leaves
D
Study area
CLe
CLg
HRAe
HRAg
a
a,c
a,c,d
b,c,e
Median
25%-75%
Non-Outlier Range
Outliers
Extremes

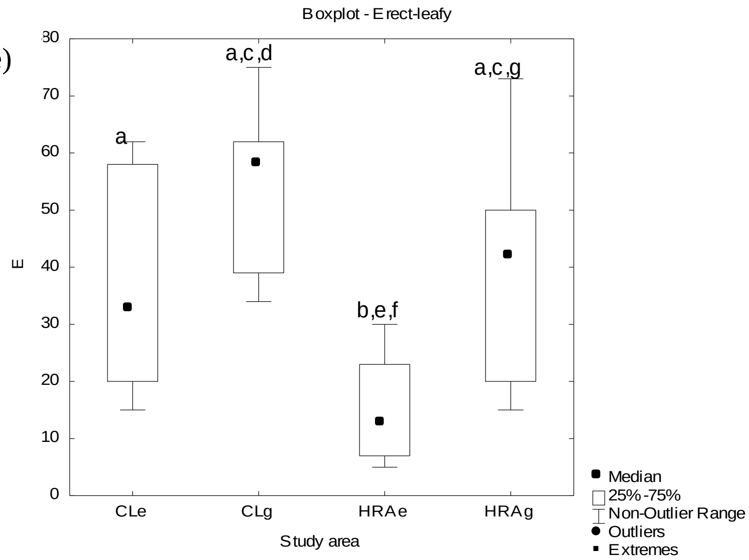
c)


Boxplot - Rocks or gravel
R
Study area
CLe
CLg
HRAe
HRAg
a
a,c,d
a,c,e
b,d,f

d)


Boxplot - Bare soil
BS
Study area
CLe
CLg
HRAe
HRAg
a
b,c,e
a,d,g
a,f,g
Median
25%-75%
Non-Outlier Range
Outliers
Extremes

e)


Boxplot - Erect-leafy
E
Study area
CLe
CLg
HRAe
HRAg
a
a,c,d
b,e,f
a,c,g
Median
25%-75%
Non-Outlier Range
Outliers
Extremes

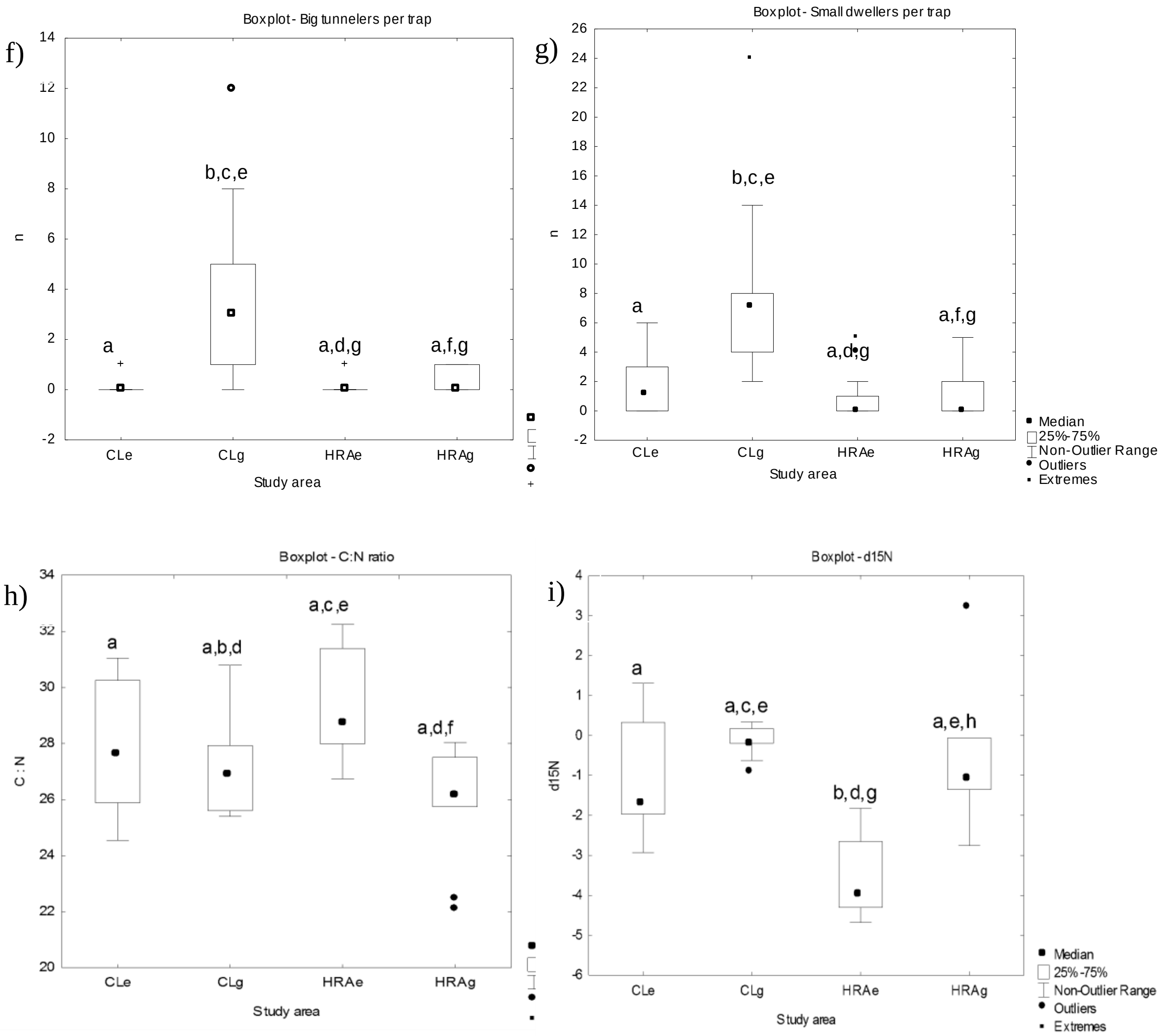
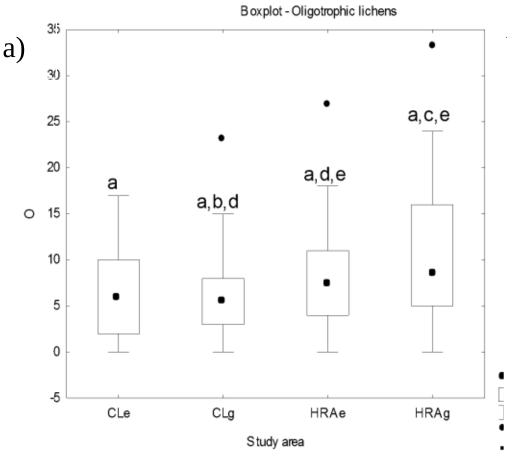
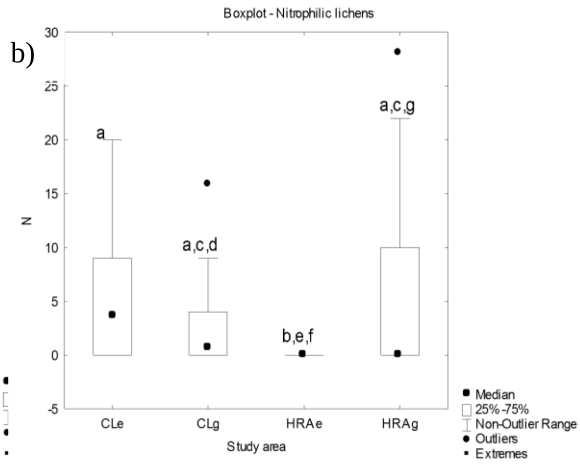
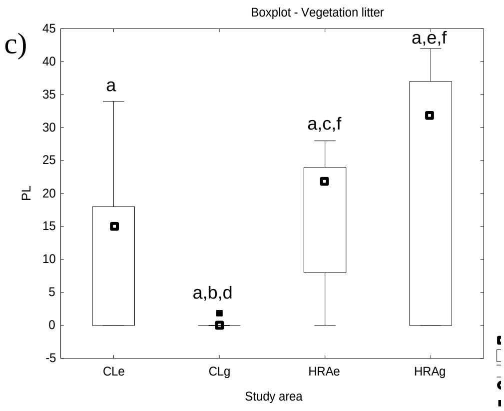


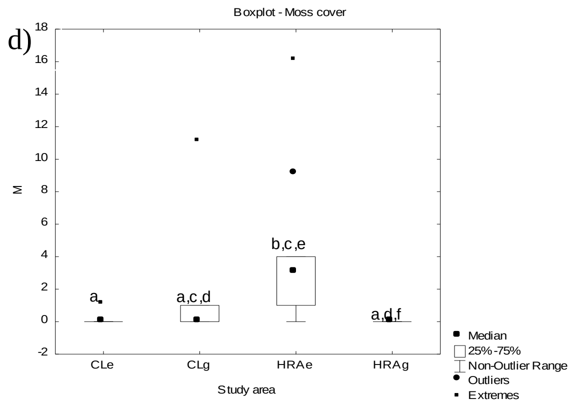
Fig. 4.4 - Boxplots for the frequency of mesotrophic lichens (a), dead tree leaves (b), rocks or gravel (c), bare soil (d), erect leafy (e), big tunnelers per trap (f), small dwellers per trap (g), C:N ratio (h), and δ15N (i) . Different letters indicate statistically significant difference (Mann-Whitney's U test; p values in Table A, Appendix III). "e" is for grazing excluded, "g" for grazed.

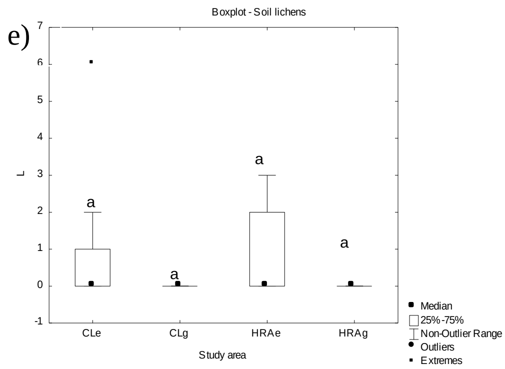
In the third group, we could find the indicators frequency of oligo- and nitrophilic lichens, vegetation litter, moss cover and soil lichens (see Fig. 4.5). The isotopic discrimination of 13C and number of big dwellers per trap were also included in this group.

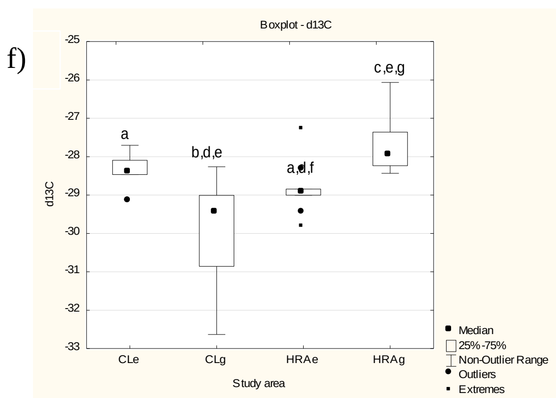

a)
Boxplot - Oligotrophic lichens
O
Study area
CLe
CLg
HRAe
HRAg
a
a,b,d
a,d,e
a,c,e


b)
Boxplot - Nitrophilic lichens
N
Study area
CLe
CLg
HRAe
HRAg
a
a,c,d
b,e,f
a,c,g
Median
25%-75%
Non-Outlier Range
Outliers
Extremes


c)
Boxplot - Vegetation litter
PL
Study area
CLe
CLg
HRAe
HRAg
a
a,b,d
a,c,f
a,e,f


d)
Boxplot - Moss cover
M
Study area
CLe
CLg
HRAe
HRAg
a
a,c,d
b,c,e
a,d,f
Median
25%-75%
Non-Outlier Range
Outliers
Extremes


e)
Boxplot - Soil lichens
L
Study area
CLe
CLg
HRAe
HRAg
a
a
a
a
Median
25%-75%
Non-Outlier Range
Outliers
Extremes


f)
Boxplot - d13C
d13C
Study area
CLe
CLg
HRAe
HRAg
a
b,d,e
a,d,f
c,e,g
Median
25%-75%
Non-Outlier Range
Outliers
Extremes

g)

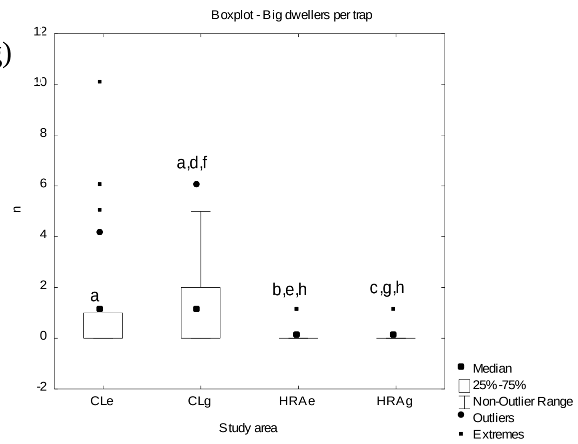
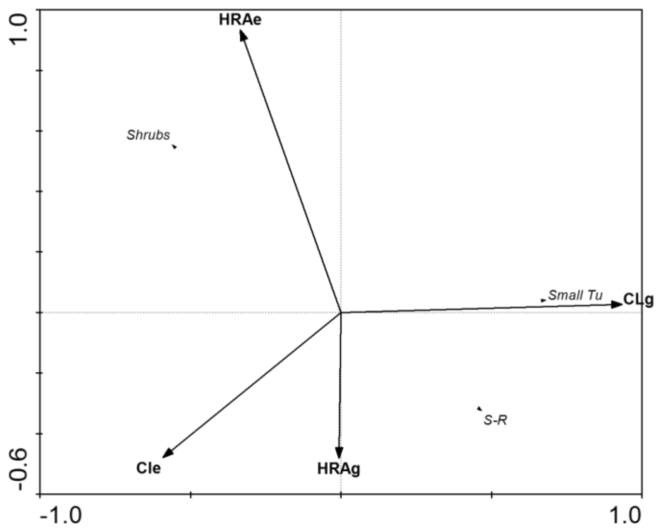


Fig. 4.5 - Boxplots for the frequency of oligotrophic (a) and nitrophilic lichens (b), vegetation litter (c), moss cover (d), soil cover (e), δ13 (f), and number of big dwellers per trap (g). Different letters indicate statistically significant difference (Mann-Whitney's U test; p values in Table B, Appendix III). "e" is for grazing excluded, "g" for grazed.

4.3 Index of Ecological Integrity

The proposed IEI revealed a good separation of grazed and grazing-excluded treatments, consistent for both study areas (Fig. 4.6-a). When accounting for the effect of several covariables this trend was maintained (Fig. 4.6-b).

a)



b)

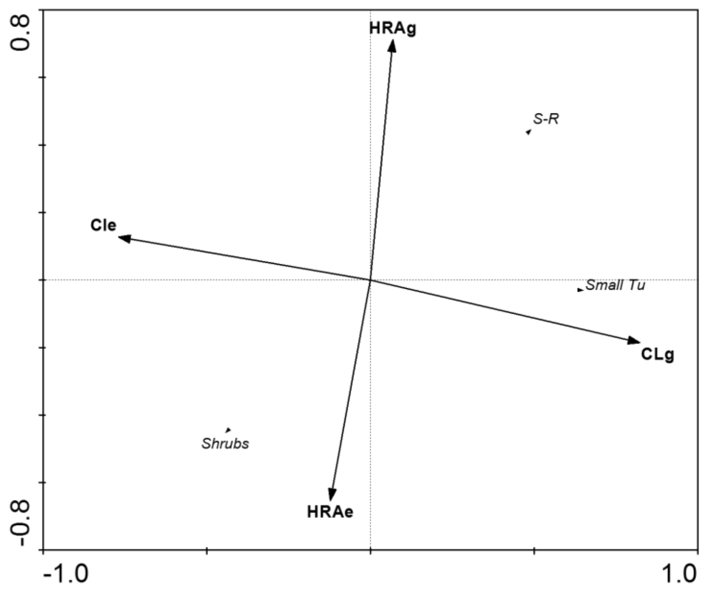


Fig. 4.6 – (a): Two first axis of an RDA with the selected indicators: semi-Rosettes, shrubs and small tunnelers, considering study areas and treatment as environmental variables. 45.0% and 2.6% variance was explained by the first and second axis, respectively. (b): partial RDA considering as covariables tree density, annual potential solar radiation and slope. "e" is for grazing excluded, "g" for grazed. 40.4% and 1.9% variance was explained by the first and second axis, respectively. (The lines of the arrows of the species were removed for graphical clarity)

The lowest values of the IEI index were associated to more impact of grazing. There were statistically significant differences observed among the grazed and the grazing-excluded treatment, for both study areas. Comparing all sampling sites, taking into consideration the impact of grazing sampling sites were ordered as HRAg, CLg, CLe, HRAe (from higher to lower impact) (Fig. 4.7).

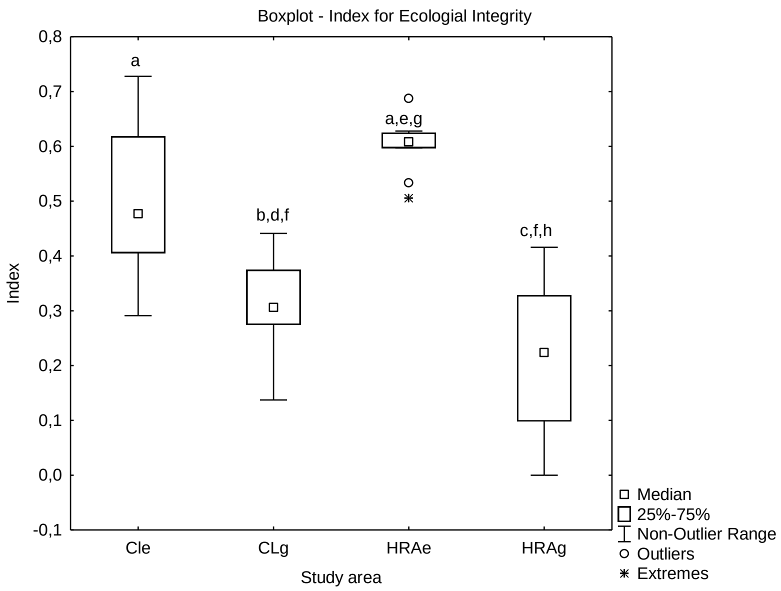


Fig. 4.7 – Boxplot of the Index of Ecological Integrity for sampling sites. The lowest values were associated to more impact of grazing, the letters indicate statistically significant difference (Man-Whitney's U test; p-values in Table 2, below). "e" is for grazing excluded, "g" for grazed.

Table 2 - p-values for the Mann-Whitney's U test performed for the boxplot in Fig. 4.7.

	CLe/CLg	CLe/HRAe	CLe/HRAg	CLg/HRAe	CLg/HRAg	HRAe/HRAg
Index	**0,008072**	0,157705	**0,002680**	**0,000412**	0,185327	**0,000412**

5. Discussion

This study provided an innovative perspective on the development of ecological indicators for the effect of grazing on cork-oak woodlands, by combining several indicators from different ecological groups and by calculating an index for measuring the impact of grazing at the ecosystem level.

Although this multi-taxa approach is rather uncommon in literature, it provided not only a measure of the impact of grazing on species but also the opportunity of understanding the impacts of grazing on ecosystem functioning, e.g. on the nitrogen cycle. Due to its integrative perspective the use of the Index of Ecological Integrity, rather than indicating a simple pressure (e.g. reflecting the amount of cattle), it reflects the effects of grazing pressure on the ecosystem.

This work was possible by selecting the most responsive indicators for the effects of grazing, looking at communities' organization, functional groups and leaves isotopic composition, and using them to calculate an integrative index.

5.1. Selection of variables as indicators for the effect of grazing

5.1.1 Communities analysis

In the light of the ordination analyses of species, unexpectedly there was a separation of the study areas but not of treatment (grazing regime). This means that the composition of the communities of lichens and coprophagous beetles are very different between study areas and their variation is probably more indicative of site-specific dynamics or of the prevailing influence of macroclimate conditions. Therefore, species ordinations should not be used for the purpose of indication of grazing impact.

5.1.2 Functional groups and isotopic composition

Looking at functional groups and tree leaves isotopic composition allowed the selection of the most responsive indicators: frequency of shrubs, frequency of semi-rosettes and abundance of coprophagous beetles. These were included in the "first group" of indicators (variables which separate treatments in both study areas) (Fig. 4.3). In general this result is supported by other studies on the effect of grazing on these groups (Braga et al., 2013; De Bello et al., 2005; Peco et al., 2012). The remaining indicators were included in a second group (variables which separate treatments in one study area) or third (variables which did not separate treatments in none of the study area) group. All indicators are further discussed below.

Lichens

It is known that lichens respond to grazing intensity (Pinho et al. 2012), but their use as indicators in this case was not possible due to a non-significant effect of grazing. The mesotrophic functional group show a higher sensitivity than the oligotrophic with significant differences in CL suggesting that the non-grazed sampling sites may be showing signs of recovery. Regarding the oligotrophic functional groups, their low abundance independently of treatment at both study areas suggests that this functional group could be affected by past or nearby grazing, even at the control sites. In fact nitrophilic lichens had similar values in grazed and grazing-excluded sites in CL. This could also be reflecting the influence of atmospheric ammonia emitted by cattle to the nearby non-grazed areas. In fact, grazed and control sites are located at less than 500 m from each other, a distance that can be overcome by atmospheric ammonia (Sutton et al. 1998). However in HRA, the nitrophilic are more frequent in the grazed plots, likely reflecting the higher availability of nutrients, especially atmospheric ammonia, which is known to favour nitrophilic species (Pinho et al., 2011).

Vascular plants and soil cover

The results of shrubs and semi-rosettes are probably related to the food preferences, once animals feed on shrubs (Öckinger et al., 2006), while semi-rosettes, being smaller and more horizontally shaped, are less grazed than erect plants, which are much easier to graze (Kahmen and Poschlod, 2004).

Among the tested indicators, we also found a large number that provided a weaker but meaningful response to grazing (the “second group”). It should be noted that the grazing intensities were low in both study areas, and perhaps low enough to not trigger all the common effects of grazing. Nevertheless, the elements in this group could be used to understand the underlying dynamics altered by grazing.

The trends of dead leaves between treatments were possibly related with vegetation structure, because shrubs and higher plants could block the sweeping of the leaves by the wind.

Soil lichens showed a trend to decrease in the grazed plots, although no significant differences between any study areas were registered. It is known that trampling plays a significant role in the growth of soil lichens, which are considered a sign of lack of disturbance (Rai et al., 2011). Here, they are likely attesting to the fact that the soil under grazing exclusion is no longer stepped on.

The presence of rocks at ground surface level, which include small rocks and gravel, varied in the same way on both study areas, albeit only in HRA the difference among treatments was significant. Grazing impacts like plant cover removal or trampling may explain the differences between treatments, as animals compact the more exposed soil and erode it to an extent where rocks show up on the surface. The resulting interrill erosion is correlated with the presence of rock and gravel at the soil surface (Gutierrez et al., 1996). The more significant results in HRA could be the soil nature (schist and greywacke), contrasting to the sandy nature of the soils in CL. Also the higher slope in HRA can be contributing to the more significant results in HRA, with the slope contributing to the speed the water gains on the surface, consequently exposing rocks more frequently.

When comparing the weight of both livestock species in this study, as well as the soil characteristics, it should not be surprising how much bare soil was found in the grazed area of CL vs. the grazed area of HRA. This is valid when comparing the two study areas, but also when the focus is on the treatment. Naturally, the difference in the livestock behaviour and management will be reflected on a local scale, with the impact of cattle being much more noticeable on CL (in which the difference between grazed and grazing-excluded is higher) than on HRA (where sheep, much lighter, will not exert such an obvious impact).

Erect leafy plants may be the first sign of recovery from grazing. The plant height had been increasing for more time in HRA than in CL, which can help to understand the bigger disparity, at treatment and study area levels.

Some of the tested indicators resulted in non-significant or non-coherent trends. Litter and dead leaves showed an opposing trend in the two study areas. In CL, grazing-excluded sites presented higher values than grazed one, which are near zero. On the other hand, in HRA, more litter and dead leaves were found in the grazed areas. This can be due to cattle consuming most of the plants in CL and the fact that the grazing-excluded sites have evolved to shrubland, with higher leaves production. In HRA, the extensive sheep grazing is not enough to deplete the biomass in the same way than in CL.

Moss only had any relevant presence in the grazing-exclusion areas of HRA. Together with grazing intensity, the higher slopes could provide more shade and humidity retention helping moss to establish and grow.

Coprophagous beetles

It is known that coprophagous beetles respond positively to grazing and are a vital part of the ecosystem in dealing with dung (Hanski and Cambefort, 1991; Nichols et al., 2008). The fact that statistical difference was only found for a sub-set of coprophagous beetles could be due to the higher amount of small tunnelers, when compared to the other tested functional groups of coprophagous beetles.

Number of big tunnelers per trap presented a significant difference in CL but not in HRA. This could be related to the amount and spatial distribution of dung. In fact, there is higher amount of dung in CL, where it is also more concentrated in space, because in HRA sheep roam freely. It is known both that the tunnelers move large quantities of earth during the nest construction process (Mittal, 1993) and that the larger species build deeper tunnels and move larger quantities of earth. The process of bioturbation is helpful to aerate the soil and to create a healthy environment for the development of soil bacteria. Coprophagous beetles have good dispersion ability. For instance, in a rain forest, Peck and Forsyth (1982) recovered some *Onthophagus* at distances of 180 to 700m after two days. That search is more complicated on a location having a stronger heterogeneity of resources, which is the case in HRA. Moreover, in HRA the herds are more mobile and produce lesser quantities of dung. Thus, it should be harder for the insects to immediately find and feed on them.

Isotopic and elemental analysis

Carbon/Nitrogen ratios decrease in the grazed sites on both study areas, although only significantly in HRA (see Fig. 4.4-h). This increase could reflect the higher availability of nitrogen in the grazed areas, due to more nitrogen input by dung and urine deposition. Higher Carbon/Nitrogen can also denote a slower rate of decomposition in the grazing-excluded sampling sites (Frank et al., 1995). Both δ13C and δ15N did not show a clear response to grazing, showing inconsistent trends. This should indicate that the source of variation is beyond the effect of grazing and is rather explained by other environmental factors.

Leaf δ13C values reflect the variations and relationship among plant carbon and water relations and can act as integrators of whole plant function (Dawson et al., 2002), being an useful indicator for the intrinsic water-use efficiency (WUE) (Dawson et al., 2002; Farquhar and Richards, 1984; Henderson et al., 1998). Among the factors that could influence δ13C we can find soil moisture (Ehleringer and Cooper, 1988; Ehleringer, 1993; Korol et al., 1999; Stewart et al., 1995), atmospheric humidity (Comstock and Ehleringer, 1992; Madhavan et al., 1991; Panek and Waring, 1997) and temperature (Panek and Waring, 1997; Welker et al., 1993) and nitrogen availability (Condon et al., 1992; Guehl et al., 1995; Högberg et al., 1993). The results suggest that trees in control sites in CL and HRA are in similar conditions, and become more stress in HRA but less stressed in CL. The lack of agreement suggests that this result is not related to the effects of grazing.

As for δ15N, both urine (wet) and dung (dry) deposition are related to an increase in its values, as demonstrated by Skinner and colleagues (2005). Elevated foliar δ15N can reflect an increase in soil N availability (Craine et al., 2009). The lack of a statistically significant difference in CL is probably related to a shorter exclusion period than in HRA, where, as stated in Methods, the plots have not been grazed for at least 20 years. It can also be related to the deposition of nitrogen incoming from the nearby grazed areas. Additionally, the amount of dung excreted by cattle is larger and more concentrated than sheep’s. So, its permanence in the soil will be more prolonged. The livestock management regime may have an influence, once the same sampling sites are visited every year by the sheep in HRA, while some years after the cattle stays on the same plot, that area is excluded from grazing to recover. In addition, sandy soils have lower contents of organic matter and total N than soils with heavier textures (Haynes, 1986). Nitrogen content (%) did not show a statistically significant difference in CL, although it did for HRA. This may be explained by the fact that a rotation system of grazing and exclusion that may not have given long enough to recover from the grazing impacts, in the case of the grazing-restricted plots. The difference between the two study areas / livestock species could be suppressed by the short period of recovery, since nitrogen stays on the soil for some time and the plot was only closed in 2008.

5.2 Index of Ecological Integrity

The integration of the results of the best indicators originates an index showed a gradient that classified study areas from the most to the least impacted. This can assist in future prioritization of restoration efforts, in case of limitation of resources. In crescent order, starting from the least impacted, there was HRAe. The justification for this lies in the fact that this space is lacking any disturbance for many years, and among these sampling sites, the sheep have not been present for more than 20 years, as referred above. Following HRAe, it was CLe, which is excluded to cattle for c. 5 years (since 2008). Some indicators show (for instance, δ15N and nitrophilic lichens), however, that the history of this study area may still be playing a relevant role.

Finally, the grazed sampling sites in CL are less impacted than the ones in HRA. Although the impact by cattle should be higher than by sheep, it should be considered how the management regimes are different in these two study areas. CL has rotation system that keeps the cattle in the same place for some time, but it removes it afterwards, for land recovery. This removal does not happen in HRA: sheep keep grazing the same space year after year, for many years now, and are only moved after it appears the resources on that area are too scarce for them to feed properly in the remainder of the season. This sustained impact on the land is, therefore, visible through this analysis, bringing to light a less obvious, but relevant aspect. Besides the livestock species, the climate is also different, having its effects. Being drier and having more temperature extremes, any stress put on HRA has an increased impact.

In conclusion, an evaluation of both the livestock species and the location of the grasslands are important to balance and mitigate the impacts, so that a system may remain stable. As seen here, this is not always easily deduced by common sense, and a careful analysis, using this Index of Ecological Integrity is advisable.

5.3 Ecosystem functioning

Grazing impacts

For the grazed sampling sites, there is an evident change in quantity and quality of resources available for plant decomposers, as other research also points out (Castro, 2008; Wardle and Bardgett, 2004). The shrub results are another measurement supporting that this phenomenon is taking place.

The elimination of soil cover tends to occur with trampling and consumption by livestock. However, McIvor and others (1995) demonstrated that even a low soil cover like 30-40% can reduce runoff and soil loss to minimal levels. This source of impact is, then, a function of intensity, rather than inevitable. Molinillo and colleagues (1997) suggests that, to minimize grazing effects, patches of dense shrub cover and grazing meadows is the best method for pasture conservation and forage production. In this study, this management option is accomplished in both study areas, with more patchiness (i.e. smaller patches) in HRA, which is adequate considering the nature of impact of sheep, and the need of cattle to have more forage resources and space.

Trampling may cause soil compaction (Gutierrez et al., 1996), vascular plant community alteration (Belnap, 1998) and exposure of more erodible soil (Herrick et al., 2009). Despite being one of the worst impacts, it also stands as one of the biggest differences among the two livestock species, once trampling by sheep does not appear to cause a great impact on grasslands (Kahmen, 2004), something that is supported by this study as well.

Animal waste has also its effects on the soil and atmosphere. Dung and urine generate Nitrogen imbalances. Dung beetles, one of the groups of this study, may help in ameliorating the impacts and perhaps to take advantage of them, if we consider how distributed dung can act as a fertilizer in the soil by increasing the available labile N for uptake by plants through mineralization (Kazuhira et al., 1991); but also the increase in species richness of nitrophilic lichens as a consequence of an increase of atmospheric ammonia production (Ruisi et al., 2005).

Integration of the N-cycle and overall ecosystem state

Of all the activities or alternatives of exploration of the *Montado*, grazing may be the most impacting. Therefore, accounting it as the most defining factor of ecosystem health is adequate. This means the evaluation of the major impacts of grazing would be a great surrogate for the overall ecosystem state.

The main concern should now be if the set of indicators that was analysed here is actually enough to capture the variability of all areas with sylvi-pastoral use, independently of macroclimate, tree species and the intensity of grazing. The four groups can represent different ways to look at the N cycle, isolating a small part of it that reflects the function of those groups in breaking or establishing a balance in the environment in which they inhabit. Lichens reflect the atmospheric nitrogen dynamic. Leaves N composition and $\delta15N$ ratios can be interpreted in order to evaluate the conditions of the soil from which they get the N, and the condition of the air, in which different concentrations of NH_3 reveal different uses or impacts on the atmosphere. Coprophagous beetles also play an important role by dispersing or removing dung turning dung from source of disturbance of the ecosystem (since its accumulation is problematic), to a well dispersed soil fertilizer. Thus, these indicators picture distinct compartments of the nitrogen cycle, and because this cycle is important to soil fertility, resistance to erosion, provision of forage, cork oaks health, air purity, general biodiversity and much more. This makes it undeniable to attribute core value to an assessment of the overall ecosystem state.

Of course there are many other good indicators. Some for factors also addressed here, others for uncovered issues. However, this study provides proof on the width of spectrum of variability, while still being very easy and cheap to perform. Moreover, most people could be equipped with the necessary knowledge to perform the methodologies effectively and efficiently. It is naturally a good decision to implement the assessment of these groups and integrate them in a system to be put to practice and demanded regularly by managers and land owners.

5.4 Applications of these indicators and methodologies

5.4.1 Easiness of use

The fieldwork of this study was relatively fast and inexpensive. This should mean that the logistics is aligned with the best interests in the application of biological indicators. Over the course of one year, it was possible to sample four different groups on two different locations. Considering the applied protocol, even more groups could be done, if the scope of other projects so required. Additionally, once these procedures are related to trends and yearly variations, their connection to consistent monitoring demands the reutilization of all the materials, which implies the growing cost-efficiency, as time goes by. Over the course of this paper, it is discussed how the three main factors hampering the application of ecological indicators referred by Dale and Beyeler (2001) were avoided. They are (a) Monitoring programs often depend on a small number of indicators and fail to consider the full complexity of the ecosystem. (b) Choice of ecological indicators is confounded in management programs that have vague long-term goals and objectives. (c) Management and monitoring programs often lack scientific rigor because of their failure to use a defined protocol for identifying ecological indicators. For (a), it is unarguable that the broad selection of indicators from different taxonomical groups present in this work are more than enough to counter this risk. To guarantee that goals and objectives of the current project (and others that may be based on this one) are not vague (b), a lot of context is provided from several points-of-view, ensuring that there is an adaptation of the monitoring to the specific program that uses these indicators. As for the last point (c), again, the research and analysis provided here may constitute solid guidance for future works, in order to stop this from becoming a reality; although it was not the main focus of this project, it is possible to understand through the successes and failures in the adequacy of each indicator, how the process of sampling should occur, or be modified in relation to the one applied here. Further analysis is always presented throughout the paper to avoid a situation like this.

5.4.2 Application at a Small scale - Human impacts management (property managers)

As seen before, it can be very important to consider the abiotic factors of the landscape to know how it will react to grazing by each livestock species. So the first advice for *Montado* management, regarding the pastures, is to study them, ideally the tree leaves isotopic composition, in order to understand the state of the ecosystem, prior to any major decision. Additionally, lichens may help in understanding the history of disturbance of the area, once they reflect change over time. Lichens may be even more revealing in situations where the land may suffer the effects of atmospheric pollution, near dirt roads with some traffic or other sources of dust, and in cases in which there was grazing before (for instance, by a previous land owner). Assessing the dung beetle populations may be interesting, just to test the added resilience that will provide more flexibility and mitigation of impacts if there is beetle biodiversity to accommodate the increase in pressure that grazing will cause. This can be seen as an evaluation on something that may tranquilize the property owners or land managers, once it decreases the risk of the investment they are making. Each land has its own plant community. Knowing that community can lead the manager to predict many things, like the forage resources the livestock will have, or how the community (and with it, the soil) will respond to grazing, once the issue is studied and in most cases can be standard.

So, why is it also relevant to keep the regularity and scope of the monitoring procedure, as was here presented? Investment on *Montados* can be both expensive and profitable.

Any tendencies evolve slowly and, if carefully studied, can be predicted in time. A new cork oak tree can take decades to be ready to be harvested and it has to be healthy all along to produce good quality cork. These two major issues make a close monitoring of the ecosystem a must, not an option. Protecting the land and its ability to be explored, to be economically viable, to help feeding people in a very sustainable and healthy way and to maintain other great values like biodiversity, aesthetics and the leisure activities it may provide demands this degree of attention.

5.4.3 Large scale - Decision-makers and public interest

Research conducted may provide decision-makers and the general public with new sources of scientifically-based and easily usable information to support land management decisions. Through using the proper biological groups and statistical analysis, governmental or non-governmental organizations can inform the citizens of the measures and results of the strategies and policies being applied.

Ecological indicators are a relevant component of the larger set of environmental indicators used to understand man-related changes imposed to ecosystems. Ecological indicators are however not the only tools. They are now being coupled with social response indicators to offer a combined view of the transformation of landscapes. In fact, it is possible to include the indicators used in this work to broader, larger-scale on-going initiatives (e.g. OECD, USEPA, LTER). It is by understanding how to scale-up the use of ecological indicators that the ecological theory may be further developed. Scaling-up can offer support for increasing ecological awareness and understanding of the general public.

As mentioned, the current project took place in two LTER-*Montado* sites, and intends to be a contribution to help boosting the participation of Portugal in LTER-Europe large network of countries and professionals, working together to gather data at very large scales. The potential for application of the studied indicators of grazing effects may help to understand the major trends in a large scale and over a long period of time. That is one of the most important missions of LTER networks. It is, therefore, of the utmost importance that strong scientifically-based knowledge is developed to understand land use trends and impacts, and support decisions on land management.

6. Conclusions

There are three main conclusions standing out from this paper. The first is the identification of a set of indicators useful to assess grazing effects in Mediterranean areas. The second refers to the analytical approach, that showed that boxplots and Mann-Whitney U tests are adequate tools to ilustrate grazing effects in different areas, and integrative indexes (such as the proposed Index of Ecological Integrity) are useful for a general understanding of the system condition. The third conclusion, is the potential of application of such approach both at small and large scales.

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Perspectives and Outreach

In this thesis, an application of various categories of ecological indicators was tested in two study areas submitted to different grazing regimes. This work went beyond the traditional comparison of species richness and abundance. The process included the need for the selection of relevant indicators, the practicalities of the sampling design and adequate statistical testing. The significance of the results obtained at local scale raise the question of how broad can be the application of the studied ecological indicators.

In order to fully understand this study potential, we can frame it in ongoing continent-sized projects. The study-cases include the United States of America, though a report by the EPA, and Europe, focusing on the OECD environmental strategy and a network of cooperation between professionals of different countries (LTER).

In the United States, the USEPA Science Advisory Board has provided, in 2002, a good example on how ecological indicators may be applied at different scales, ranging from local to national. “A Framework for Assessing and Reporting on Ecological Condition” (Young and Sanzone, 2002) has the primary purpose of designing a system to guide the process of assessing the ecological condition at various spatial scales. It focuses on which ecological attributes to measure and their aggregation as a tool to understand ecological integrity. The current study shares some aspects with the items in the report. For instance, the “Biotic Condition” attribute, indirectly, has a strong relationship with “Landscape Condition”, by using “Landscape Composition” (one of its categories) to explain the variations on some of its indicators, and with “Chemical and Physical Characteristics (Water, Air, Soil, Sediment)” once foliar analysis partly reads the dynamics of “Nutrient Concentrations”, and can be generalized to a larger spectrum of the ecosystem. As a matter of fact, all the attributes are reflected on the selected indicators, because they reflect the ecosystem variability.

In Europe, the report "Towards Sustainable Development", OECD (2001) states that their environmental indicators are: i) "regularly used in environmental performance reviews"; ii) "a valuable way to monitor the integration of economic and environmental decision making, to analyse environmental policies and to gauge the results"; and further iii) "contribute to follow-up work on the OECD environmental strategy and to broader objective of reporting on sustainable development". This is one the best examples on how indicators can serve a very large-scale purpose on informing about the environment and to track progress on sectors like, for instance, agriculture, energy and transport.

For the Long-Term Ecological Research (LTER) Network, ecological indicators to monitor changes in the ecosystems structure and functioning are a key issue. The network started in the USA in the 1980's and was established in Europe in 1993. As of 2011, 22 European countries were involved in LTER Europe, Portugal being one of those. ILTER (International LTER network) unites and coordinates both European and American partners, and all the other adherent countries in the world.

LTER is transitioning to LTSER (Long-Term Socio-Ecological Research). Here, work on human activities and impacts is of extreme relevance to explore human behaviour and response to change for more ecologically sound alternatives. However, not regarding social and economic aspects of new management strategies could be a mistake that would compromise the effectiveness of any programme. To prevent that, the most convincing way is showing proof of future tendencies and consequences. Indicators are the ideal manner to bridge an understanding, by being an independent and unbiased clarification of environmental changes.

Following up this study, it would be a good idea to design a comprehensive procedure of the application of ecological indicators nationally, for instance for all the Portuguese

LTER sites, with a plan for sampling for the next 5-10 years. The limitations of the presented work that would be ideally avoided in the new project would be a more careful analysis of the environmental factors affecting each location and increasing the sampling effort to make the distinction among treatments more clear. A higher number of locations should also be sampled. It could also be interesting to subject the same or another set of ecological indicators to another issue, like fires, restoration programmes, land change (agriculture to abandoned land, and vice-versa).

Ecological indicators can, therefore be applied to many more situations, in order to answer to the main environmental issues. Once there is basic knowledge of the groups that are to be tested, it is possible to derive interesting conclusions about the trends of a landscape.

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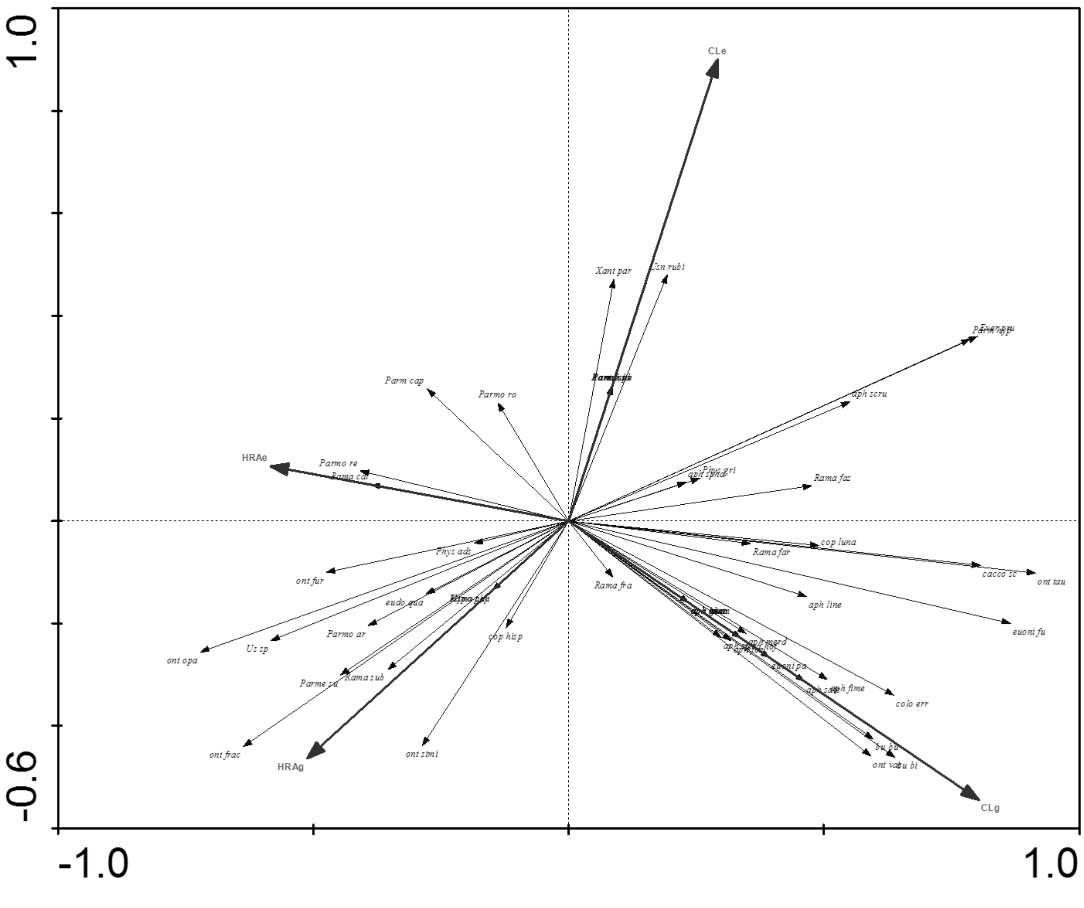
Appendixes

Appendix I - Study areas, photos taken during the sampling periods. Companhia das Lezírias: grazed area (a), Herdade da Ribeira Abaixo: grazing-excluded area (b).

a)

b)

Appendix II - Ordination diagram (RDA) with the coprophagous beetles having a representation of more than 20 captured individuals and lichens species, and the study areas separated by treatment as environmental variables. “e” is for grazing excluded, “g” for grazed. 59.0% and 6.1% of the species variance were explained by the first and second axis, respectively.



Appendix III – Tables with p-values from the second (a) and third (b) groups of indicators (Figs. 4.4 and 4.5)

a)

Indicator	CLe/CLg	CLe/HRAe	CLe/HRAg	CLg/HRAe	CLg/HRAg	HRAe/HRAg
Mesotrophic lichens	**0,022361**	0,057460	**0,002892**	0,604839	0,147374	0,082358
Dead leaves	0,093399	0,353838	0,250999	0,309880	0,507801	**0,007077**
Rocks and gravel	0,309880	0,596242	0,077390	0,093399	0,658844	**0,013419**
Bare soil	**0,000574**	0,353838	0,859819	**0,000412**	**0,030511**	0,507801
Erect leafy	0,057632	**0,007077**	1,000000	**0,000412**	0,077390	**0,007077**
Big tunnelers	**0,000003**	0,787985	0,401794	**0,000002**	**0,000009**	0,261366
Small dwellers	**0,000012**	0,367217	0,447658	**0,000002**	**0,000005**	0,849441
C:N	0,479929	0,111962	0,289316	**0,027276**	0,596242	**0,001998**
δ15N	0,216374	**0,003569**	0,289316	**0,000412**	0,133321	**0,001998**

b)

Indicator	CLe/CLg	CLe/HRAe	CLe/HRAg	CLg/HRAe	CLg/HRAg	HRAe/HRAg
Oligotrophic lichens	0,559231	0,264327	0,103890	0,064596	**0,024626**	0,477920
Nitrophilic lichens	0,151548	**0,000168**	0,487139	**0,000903**	0,923442	**0,027087**
Vegetation litter	0,077390	0,426777	0,133321	**0,001998**	**0,027276**	0,200412
Moss cover	0,658844	**0,013419**	0,452913	0,063691	0,250999	**0,006194**
Soil lichens	0,250999	0,723932	0,250999	0,122278	1,000000	0,122278
δ13C	**0,021685**	0,157705	**0,042261**	0,111962	**0,001479**	**0,004718**
Big dwellers	0,763745	**0,007508**	**0,003793**	**0,005110**	**0,003095**	0,787985

Appendix IV – Details on the Index of Ecological Integrity construction process

Step 1 – Determining the best indicators. Boxplots in Fig. 4.3

Step 2 – Ordination analysis (first an RDA, then a partial-RDA) with these three main indicators (Fig. 4.4).

Step 3 – Retrieving the "scores" (coordinates in the graph)

Small Tun	-0,808
Shrubs	0,332
S-R	-0,739

Step 4 – Using those scores and crossing them with the original data

Totals	Small Tun	Shrubs	S-R
P1S1	32	34	0
P1S2	89	37	1
P1S3	76	26	1
P2S1	119	2	7
P2S2	68	0	0
P2S3	127	4	2
P3S1	60	1	5
P3S2	78	14	2
P3S3	127	8	0
P4S1	169	0	0
P4S2	221	0	4
P4S3	172	0	7
P5S1	195	0	9
P5S2	167	1	11
P5S3	205	0	20
P6S1	141	0	18
P6S2	162	0	11
P6S3	184	0	8
P7S1	195	39	9
P7S2	86	33	2
P7S3	181	40	0
P8S1	68	34	2
P8S2	100	28	0
P8S3	151	23	3
P9S1	65	18	1
P9S2	78	34	2
P9S3	34	30	1
P10S1	139	25	14
P10S2	71	8	12
P10S3	59	0	8
P11S1	140	19	6
P11S2	113	11	6
P11S3	156	0	11
P12S1	115	2	22
P12S2	172	4	19
P12S3	123	3	20

Each value above is multiplied by the "scores" in step 3, originating the table below.

Small Tun	Shrubs	S-R
-0,78352	0,268762	0
-0,72638	0,292476	-0,0176

-0,74888	0,205524	-0,0176
-0,73963	0,01581	-0,12317
-0,77386	0	0
-0,72777	0,031619	-0,03519
-0,808	0,007905	-0,08798
-0,77807	0,110667	-0,03519
-0,73297	0,063238	0
-0,73812	0	0
-0,72003	0	-0,07038
-0,72383	0	-0,12317
-0,71618	0	-0,15836
-0,61897	0,007905	-0,19355
-0,69017	0	-0,3519
-0,70326	0	-0,31671
-0,71139	0	-0,19355
-0,73237	0	-0,14076
-0,79576	0,308286	-0,15836
-0,808	0,260857	-0,03519
-0,808	0,31619	0
-0,78491	0,268762	-0,03519
-0,79216	0,221333	0
-0,80268	0,18181	-0,05279
-0,70027	0,142286	-0,0176
-0,7878	0,268762	-0,03519
-0,78491	0,237143	-0,0176
-0,80223	0,197619	-0,24633
-0,808	0,063238	-0,21114
-0,808	0	-0,14076
-0,808	0,15019	-0,10557
-0,79395	0,086952	-0,10557
-0,77807	0	-0,19355
-0,808	0,01581	-0,3871
-0,78518	0,031619	-0,33431
-0,75291	0,023714	-0,3519

Step 5 – The three values from each line of the table above are then summed (table below)

Totals	**Index**
P1S1	-0,51475
P1S2	-0,4515
P1S3	-0,56095
P2S1	-0,84699
P2S2	-0,77386
P2S3	-0,73134
P3S1	-0,88807
P3S2	-0,7026
P3S3	-0,66973
P4S1	-0,73812
P4S2	-0,79041
P4S3	-0,847
P5S1	-0,87454
P5S2	-0,80462
P5S3	-1,04207
P6S1	-1,01997
P6S2	-0,90494
P6S3	-0,87314
P7S1	-0,64583
P7S2	-0,58233
P7S3	-0,49181
P8S1	-0,55134
P8S2	-0,57082
P8S3	-0,67366
P9S1	-0,57558
P9S2	-0,55423
P9S3	-0,56537
P10S1	-0,85094
P10S2	-0,9559
P10S3	-0,94876
P11S1	-0,76338
P11S2	-0,81257

P11S3	-0,97162
P12S1	-1,17929
P12S2	-1,08787
P12S3	-1,0811

Step 6 – The minimum value is summed to the entire table, so the new minimum is zero.

Totals	**Ind**
P1S1	0,664532
P1S2	0,727783
P1S3	0,618336
P2S1	0,332298
P2S2	0,405427
P2S3	0,447941
P3S1	0,291214
P3S2	0,476688
P3S3	0,509552
P4S1	0,441167
P4S2	0,388873
P4S3	0,332286
P5S1	0,304747
P5S2	0,37467
P5S3	0,137214
P6S1	0,159312
P6S2	0,274347
P6S3	0,306149
P7S1	0,533457
P7S2	0,596952
P7S3	0,687476
P8S1	0,627943
P8S2	0,608462
P8S3	0,505625
P9S1	0,60371
P9S2	0,625057
P9S3	0,613919
P10S1	0,328343
P10S2	0,223381
P10S3	0,230524
P11S1	0,415905
P11S2	0,366719
P11S3	0,207664
P12S1	0
P12S2	0,09142
P12S3	0,098186

Step 7 – The categories of CLe, CLg, HRAe, HRAg were assigned and a final boxplot was designed (Fig. 4.7).

Appendix V – Bibliographic revision tables

The following tables correspond to a bibliographical revision that resulted from the study of the groups included in this project. Most of what is referenced in these tables was used for the writing of the article, and the ones that were not can be useful for future works, including the same indicators. Besides being about the groups focused on the thesis, they are also related to grazing or its effects.

a) Lichens

Indicator	Environmental Pressure and Reference	Environmental Consequence	Interpretation
Lichens' functional groups	Land use gradients; Pinho et al. 2012	Changes in functional groups observed along the land-use gradient	Increased concentration of ammonia is expected even with a small number of animals
	Land use response; Brown 1992; Ruisi et al. 2005	Change in composition of lichen floras	Both natural hypertrophication from grazing animals (wind-blown dust from excreta and urine) and artificial hypertrophication from application of fertilizers lead to nutrient enrichment of neighboring habitats
	Land use response; Nash and Gries 1986; McCune et al. 1997; Esseen and Renhorn 1998; Gombert et al. 2004; Frati et al. 2006; Geiser and Neitlich 2007; Ruisi et al. 2005	Lichens are good bioindicators of forest ecosystem health	Being very sensitive to environmental changes
Lichens' species richness	Woodland management; Van Herk 1999, 2001; Wolseley et al. 2006; Pinho et al. 2008, 2009; Aragon et al. 2010	Decrease in lichen richness and also total cover (intensively managed dehesas) may relate to the management regimes	Lichens are particularly sensitive to eutrophication of bark by atmospheric deposition, specially inorganic contaminants (ammonia) of agricultural activities and livestock management
	Land use gradients; Pinho et al. 2012	Abundance of sensitive species decreased	With increasing LUI but without changes in richness
	Land use gradients; Pinho et al. 2012	Observed total species richness increase	Increase of nitrophytic species while at the same time oligotrophic species richness was maintained
Less oligotrophic species	Land use gradients; Pinho et al. 2009, 2011, 2012	Decrease in oligotrophic species richness and abundance until complete disappearance	Increasing cattle density

	Land use gradients; Vilsholm et al. 2009; Pinho et al. 2012	Decrease in frequencies of oligotrophic lichen species on twigs	Increasing nitrogen, but a very low decrease in richness of those species
Less oligo- and mesotrophic species	Pollution response; Pinho et al. 2011	Mesotrophic species can be similar in response to the oligotrophic species	The LDVmeso value uniformly decreases with increasing [NH3]atm, with no evidence of a maximum score at intermediate [NH3]atm
Less oligotrophic, more nitrophitic species	Woodland management; Ruoss 1999; van Herk 1999, 2001; Wolseley et al. 2006; Pinho et al. 2008, 2009; Aragon et al. 2010	Change in lichen communities from oligo- to nitrophytic species	Atmospheric ammonia, direct or indirect impact by changing bark pH
	Woodland management; Fuertes et al. 1996; Hedenås and Ericson 2004; Motiejûnaitë and Faùtynowick 2005; Aragón et al. 2010; Aragon et al. 2010	Change in epiphytic lichen composition increasing the nitrophytic and crustose species (less-managed forest)	Caused by livestock management and the canopy structure. In the agricultural and livestock landscapes, these species may be favored by increased deposition of nutrient-bearing dust
	Woodland management; Del Guasta 1994; Gilbert 1976; Jürging 1975; Loppi 1996; Paoli et al. 2012	Shift from lichen communities dominated by Flavoparmelia caperata and Parmotrema perlatum towards communities dominated by Xanthoria parietina and Physcia adscendens	When damage is not so intense, the general picture of the epiphytic lichen vegetation is influenced by sources of dust such as quarries, cement plants or dirty roads
	Pollution response; Loppi and De Dominicis 1996; Paoli et al. 2012	Promotion of nitrophytic lichen vegetation	The effect of dust on lichen communities is similar to that of ammonia emissions
	Land use gradients; Gaio-Oliveira et al. 2001; Pinho et al. 2012	Growth of lichens which are tolerant to nitrogen	Atmospheric ammonia at low concentrations might have a fertilizer effect
More nitrophitic species	Land use gradients; Branquinho et al. 2008; Pinho et al. 2008; Pinho et al. 2012	Increase of nitrophytic species frequency	Dust has a negative impact on lichen physiology
	Pollution response; Ruisi et al. 2005; Frati et al. 2007; Pinho et al. 2011	Increases in nitrophytic species	Increase in [NH3]atm
	Land use response; Pirintsos et al. 1998; van Herk 2001; Ruisi et al. 2005	Grazing induces nitrophytic lichen flora (Greece)	Even if dust may play an important role in promoting nitrophytic species on Quercus is closely dependent on NH3 concentrations. However, these features are governed by a high bark pH caused by the alkaline properties of NH3 rather than by increased nitrogen availability
	Land use response; van Dobben et al. 2001; Ruisi et al 2005	Nitrophitic species variation	A positive correlation between the abundance of nitrophytic species and the bark pH, as well as the atmospheric ammonia was found (The Netherlands)
	Land use response; Loppi and De Dominicis 1996; van Herk 2001; Ruisi et al. 2005	Nitrophytic species may be promoted by dust itself and not by nitrogen	Dust could be responsible for high bark pH values
	Land use response; Ruisi et al. 2005	Stimulation of the growth of nitrophytic species	There is a “fertilizing” effect rather than depleting one on lichen vegetation due to the presence of nitrogen compounds and/or dust or other factors

b) Vascular plants and soil cover

Indicator	Environmental Pressure and Reference	Environmental Consequence	Interpretation
Grazing	Low grazing intensity, Kahmen 2004	Low grazing is more selective	Less homogeneity in landscape structure
Growth form	Grazing; Castro et al., 2010; Fernández-Alés et al., 1993; Hadar et al., 1999; Lavorel et al., 1999; Noy-Meir et al., 1989; Peco et al., 2005; Sternberg et al., 2000; Peco et al. 2012	Good response to grazing	Similar response in Mediterranean areas with history of grazing
Less bare soil	Rainfall; Gutierrez et al. 1996	Above 20% grass cover, there is less soil detachment and soil transportation	Plant cover provides protection for the soil
	Rainfall; Gutierrez et al. 1996	Plant cover above 50% did not significantly affect hydrologic processes	No signs of changes in hydrology
	Rainfall; Gutierrez et al. 1996	Above 75% plant cover, runoff is slight, while below this value, runoff increases rapidly.	Accentuated difference where there is some bare soil (CL more distinct)
	Rainfall; Gutierrez et al. 1996	The greater the percentage of grass cover and thus above-ground biomass	Greater ability to protect the soil from raindrop impacts and to offer more resistance for runoff to flow
More bare soil	Grazing abandonment; Grime 2001; Peco et al. 2012	Increse after abandonment	More competition for light, favouring tall and erect species
Less erect-leafy	Grazing abandonment; Noy-Meir et al. 1989; Peco et al. 2012	More small or prostrate species	Herbivory promotes removal of tall erect plants and survival of small and prostrate ones
Litter	Rainfall; Gutierrez et al. 1996	Influences runoff and interrill erosion properties	By not generating a soil protection layer
	Ecological gradients; Herrick et al. 2009	Contributes positively to foliar cover protection of the soil surface	Also reflects reduced grazing frequency or intensity
	Rainfall; Eldridge & Rothon 1992; Gutierrez et al. 1996	Continued use of resources leads to a reduction of vegetation cover	More grazing means less litter production and an increase of runoff and interrill erosion
Rocks	Rainfall; Wilcox & Wood 1989; Gutierrez et al. 1996	Rock and gravel cover increases	Total interrill erosion is positively correlated with rock and gravel
More rosettes	Grazing abandonment; Peco et al. 2012	Increse after abandonment	Grazing favors rosette morphology
More graminoid or erect forms	Climate gradient; Dirks et al. 2010; Sardans and Peñuelas 2013	Better soil protection; higher microbial activity and availability of nutrients	More water availability corresponds to an increase in plant-production capacity
More rosettes, less erect plants	High grazing intensity; Kahmen 2004	Rosettes out-compete erect plants	With high grazing, taller growth forms get removed more often

Plant cover	Ecological gradients; Herrick et al. 2009	Soil hydrology	Effect on the amount of water that is released to surface and groundwater after water has soaked into the soil; shade the soil; limit evaporation; also carry water from deep in the soil into the atmosphere
	Ecological gradients; Herrick et al. 2009	Wind erosion	Where widely spaced, the density of the vegetation is also important
Plants, litter, rocks, lichens, mosses	Ecological gradients; Herrick et al. 2009	Correlated with soil and site stability and hydrologic function	Protects the soil surface from raindrop impact, limiting detachment of soil particles and physical crusting of the soil surface; higher cover means there are more obstructions to water flow
Plants; lichens	Ecological gradients; Herrick et al. 2009	Soil erosion	Soil erodibility is reduced by soil organic matter; lichens and photosynthetic cyanobacteria can play an important role in stabilizing soil
Livestock species (sheep)	Grassland management; Johansson and Hedin, 1995; Ockinger et al. 2006	Less leaves and young sapling of trees and shrubs	Sheep feeding preferences
Trampling (sheep)	Grazing; Kahmen 2004	Little effect in temperate grasslands	Lack of statistically significan difference in HRA of bare soil can be explained by this
Trampling	Rainfall; Wilcox & Wood 1989; Bradford et al. 1987; Gutierrez et al. 1996	Less plant cover and more trampling increases soil compaction	strength of the soil surface and thus reduces soil detachment and particle availability
	Trampling; Belnap 1998	Trampling leads to soil compaction	Compacted soils result in: (1) less water infiltration for use by vascular plants, (2) fewer microorganisms and thus slowed plant litter decomposition and less nutrients available for vascular plants, and (3) less root penetration by vascular plants
	Trampling; Belnap 1998	Vascular plant community composition alteration	Trampling can change plant community architecture and composition, resulting in less available wildlife cover and forage
	Ecological gradients; Herrick et al. 2009	Soil disturbance	The bonds that hold soil particles together break, and the more erodible soil below is exposed

c) Coprophagous beetles

Indicator	Environmental Pressure and Reference	Environmental Consequence	Interpretation
Community composition	Grazing pressure; Fincher 1973; Hanski and Cambefort 1991; Sowig 1995; Doube 1987; Lumaret and Kirk 1987; Kingston 1977; Doube 1991, Pinero and Avilla 2004	Differences in community composition and abundance of dung insects	Abiotic and biotic factors such as soil characteristics, vegetation and predation
	Grazing pressure; Mares and Rosenzweig 1978; Orians and Paine 1983; Kerleyand Whitford 1994; Valone et al. 1994; Pinero and Avilla 2004	Regional divergence in community composition and processes	Variation in ecological and historical factors
	Landscape effect; Roslin et al. 2001	Aphodiinae community composition	At the community level, dissimilar responses to landscape composition in different Aphodius species will translate into variation, locally
	Ecological functions and services; Andresen and Levey 2004; Andresen 2001, 2002; Slade et al. 2007; Vulinec 2002; Ponce-Santizo et al. 2006; Nichols et al. 2008	Beetles and seed burial	The probability and depth of a seed’s vertical burial by a dung beetle depends on seed size, the composition of the dung beetle community and both the amount and type of dung
	NA; Lumaret et al. 1992; Hanski and Cambefort 1991	Dung amount (resource availability) seems to determine local variation in dung beetle diversity	It is known that when the grazing regime changes from sheep to cattle, both composition and abundance of dung beetles adjust to the new conditions; grazing-pressure history and management regime also seem to be critical
	NA; Hanski and Cambefort 1991	Local variation in dung beetle diversity	Number of livestock, grazing history, distance between grazed localities and rate of dung are key factors
	NA; Hanski and Cambefort 1991	Habitat specialisation can act on between-habitat to larger scales, while the low dispersal capacity can mainly determine the spatial turnover of species on a within-habitat scale	However, the degree of dependence of the whole geographic range on ecological dispersal capacity and the latter’s interdependence on habitat specialisation still remains unknown
	Landscape gradient; Numa et al. 2012	Small differences in environmental values among sampling localities can have an effect	In Numa's study, maximum difference in mean temperature: 0.7 ºC; in total precipitation: 155 mm; in elevation: 160 m were enough for local environmental variables to have a relatively high ability for explaining variation in dung beetle abundance
	Landscape gradient; Koskela and Hanski 1977; Galante et al. 1993; Numa et al. 2012	Micro-thermohygrometric conditions can influence the colonisation of dung	This climatic factor can also explain dung beetle species movements from open to closed areas during the hottest months
	Landscape gradient; Lumaret and Kirk 1987; Zamora et al. 2007; Romero-Alcaraz and Ávila, 2000; Numa et al. 2012	The landscape variation in friction values for a forest species type and the distance to patches of open or closed vegetation are important variables for explaining local abundances	Studies about habitat preferences in dung beetles in Mediterranean conditions have shown that closed vegetation characterised by dense scrubland or brushwood cover can impede the movements of individual dung beetles and the dispersion of odour cues, consequently hindering the colonisation of dung resources
	Landscape gradient; Hanski 1986; Numa et al. 2012	Local abundances can be influenced by intrinsic population processes, but also by changes in immigration and emigration rates, which are in turn conditioned by the landscape structure	Regional variation in Mediterranean dung beetle abundances may be viewed as a consequence of the impact of vegetation filters on a group of species mainly adapted to the consumption of herbivorous faeces in open biomes
	Landscape gradient; Lande et al. 2003; Numa et al. 2012	Species abundance variance	Also affected by random or demographically intrinsic effects that are unrelated to environmental characteristics of their localities and surroundings

	Grazing gradient; Lumaret and Kirk 1987; Galante et al. 1995; Lumaret 1980; Verdú et al. 2000	In Mediterranean ecosystems, vegetation cover is an important factor in local distribution of Scarabaeoidea	In brushwood holm-oak ecosystems coprophagous fauna does not show preferences for closed areas, but rather concentrate in open areas
	Landscape gradient; Montalvo et al. 1993; Montserrat 1991; Villar and Montserrat 1995; Numa et al. 2012	Grazing should not be considered as a disturbance in some ecosystems with a long history of herbivory	Controlled grazing activity of sheep could certainly favour the historical sustenance of the biodiversity of the ecosystem. A mosaic of plant communities could be supported at a low cost, alternating patches with open and dense vegetation
	NA; Hancock 1953; Olechowicz 1974; Hanski 1987	In pastures, availability of dung remains stable	Cattle produce about 12 pats per animal per day, and in one study sheep produced 0.24 dropping per square meter per day
	Ecological functions and services; Galbiati et al. 1995; Kabir et al. 1985; Bang et al. 2005; Lastro 2006; Kabir et al. 1985; Macqueen and Beirne 1975; Bang et al. 2005; Nichols et al. 2008	Dung beetles and plant biomass	Dung mixing actions by dung beetles result in significant increases in plant height, above-ground biomass, grain production, protein levels and nitrogen content
	Ecological functions and services; Nichols et al 2008	Pest control	Control of the abundance of dung-breeding hematophagic and detrivorous flies and dung-dispersed nematodes and protozoa
	Ecological functions and services; Nichols et al. 2008	Reduce fly infestations	When and where dung beetles and dung flies co-occur, fly survival tends to decline as a consequence of asymmetrical competition for dung resources, mechanical damage of eggs by beetles, and fly predation by mites phoretic on dung beetles
Ecological function	Ecological functions and services; Miranda et al. 2000; Yokoyama et al. 1991; Vulinec et al. 2007; Nichols et al 2008	Ecosystem services	Soil conditioning and nutrient recycling by dung beetles; isolation and synthesis of the chemical compounds that suppress pathogenic fungal growth on dung beetle brood balls; Secondary seed dispersal
	Ecological functions and services; Davis et al. 2004; Nichols et al. 2008	Land management and beetle populations	Natural grasslands modified for livestock pasturing offer altered vegetation density, soil temperature and moisture support – leading to range expansion for some dung beetles species
	Ecological functions and services; Nichols et al. 2008	Negative effects of dung beetles	Dung beetles have also been implicated in increasing seed mortality and dispersing pathogens
	NA; Hanski and Cambefort 1991	Grazing area of impact	The radius of influence of increased grazing does not seem to exceed 1 km or 2 km
	Land use gradient; Slade et al. 2007; Braga et al 2013	The proportion of seeds removed covaries with the amount of dung removed	But more small seeds were removed than large seeds for a given proportion of dung removed
	Ecological functions and services; Nichols et al. 2008	The transfer of freshly deposited waste below the soil surface by tunneler and roller dung beetle species	Physical relocation of nutrient rich organic material and instigation micro-organismal and chemical changes in the upper soil layers
	NA; Hanski and Cambefort 1991	The effect of habitat variety on Aphodiinae and Geotrupinae richness must be more pronounced than on that of Scarabaeinae.	As the larger areas tend to be environmentally more heterogeneous, and the rate of species increase with area differs significantly between groups, on a geographical scale
Functional groups	NA; Hanski and Cambefort 1991	Aphodiinae species are less able to disperse between neighbouring droppings than Scarabaeinae species, on a scale of 10 m (between-dropping movements)	Group differences in dispersal capacity may lead to: i) a greater contribution to dung pat richness from the group (Scarabaeinae) with greater between-dropping mobility; and ii) greater species homogeneity of this group between neighbouring sites
	NA; Hanski and Cambefort 1991	Scarabaeinae dominates α-diversity on a between-dropping scale; Aphodiinae does so on local to larger scales	The richness of communities established in each dung pat depends on the Scarabaeinae species, but the local and regional pool richness depends on Aphodiinae
	Ecological functions and services; Mittal 1993; Edwards and Aschenborn 1987; Halffter and Edmonds 1982; Lindquist 1933; Nichols et al. 2008	Tunnelers effect on the soil	Move large quantities of earth to the soil surface during nesting (bioturbation); the tunnel depth and amount of soil removed are positively related to beetle body-size
Large beetles	Land use gradient; Feer 1999; Braga et al. 2013	Larger dung beetles also bury more seeds than do smaller beetles	Despite the large-beetle assemblage being the major group responsible for ecological functions, the small-beetle assemblage is also important for the function of dung removal

	Land use gradient; Gardner et al. 2008; Dangles et al. 2012; Slade et al. 2007; Larsen et al. 2005; Braga et al 2013	Larger-bodied dung beetles are more susceptible to abundance decline in disturbed systems	These species are the most related to function loss
	Land use gradient; Braga et al 2013	Species richness of large beetles	The variable that may best explain the variation in the amount of dispersal for large seed mimics and the amoung of soil excavation
	Land use gradient; Braga et al 2013	Small beetles collectively can be able to remove large amounts of dung from the agriculture system	On the other hand, small beetles build smaller tunnels than do large beetles, therefore excavating less soil
Small beetles	Land use gradient; Slade et al. 2007; Barragán et al. 2011; Dangles et al. 2012; Braga et al 2013	The abundance of small beetles may not influence significantly any of the ecological functions measured	Small beetles have little effect on dung and seed removal. It is likely that higher species richness is related to a higher diversity of functional groups, an attribute that has been shown to be important in predicting the amount of ecological functions
	Ecological functions and services; Gillard 1967; Yokoyama et al. 1991; Nichols et al. 2008	Dung beetles prevent the loss of N through ammonia (NH3) volatilization	By burying dung under the soil surface; also enhance soil fertility by increasing the available labile N available for uptake by plants through mineralization
Nitrogen	Ecological functions and services; Yokoyama et al. 1991; Nichols et al. 2008	Dung beetles affect the nitrogen cycle	Acceleration of mineralization rates; Nitrogen volatilization and mineralization are bacteria-mediated processes, and dung beetles alter the microorganism fauna in dung pats and brood balls during feeding and nesting
	NA; Papp 1975; Hammer 1941; Hanski 1987	Dung beetle feeding habits	Coprophagous beetle and fly larvae in cow pats consume primarily undercomposed plant parts and the liquid cementing material, respectively, but coprophagous fly larvae and adult beetles may procure accessory foodstuffs by digesting microorganims found in large quantities in dung
Behaviour and feeding	NA; Hanski and Cambefort 1991	Dwellers behaviour	Aphodiinae comprise the bulk of dwellers: they eat their way through the dung, and most species deposit their eggs in dung pats without constructing any kind of nest or chamber
	NA; Hanski and Cambefort 1991	Tunnelers behaviour	Many tribes of Scarabaeidae are tunnelers: they dig a more or less vertical tunnel below the dung pat and transport dung into the bottom or the burrow
	NA; Hanski and Cambefort 1991	Rollers behaviour	Many Scarabaeidae have evolved the ultimate dung beetle skill of making a ball of dung, a transportable resource unit, and rolling it for a shatter or longer distance before burying it into a suitable spot (rollers)
	NA; Hanski and Cambefort 1991	Sheep dung and beetle distribution	Both species richness and the number of individuals are variables that show a spatial autocorrelated pattern. On the contrary, the amount of sheep dung in one locality is not spatially autocorrelated, probably because flocks follow predetermined routes or directions
Livestock species	Lobo et al. 1998; Hanski and Cambefort 1991	Cattle grazing continuity	In localities where cattle grazing are continuous in time and great in numbers, the diversity and abundance of dung beetles is remarkably higher than in recently grazed localities
	NA; Hanski and Cambefort 1991	More sheep grazing	As more sheep graze in a smaller area, dung is quickly renovated, favouring larger populations both in each site and in the surroundings
	Landscape gradient; Bokdam and Gleichman 2000; Verdú and Galante 2004; Numa et al. 2012	Grazing systems that combine cattle with smaller herbivores such as sheep, deer or rabbits can suppress scrubland and forest growth more effectively	An increase in other types of herbivores, such as rabbits, would likely generate more structurally diversified scrublands, facilitating dung beetle movements and dung colonisation, especially for species adapted to rabbit dung pellets
Management strategies	Grazing gradient; Rey-Benayas et al. 2008; Bugalho et al 2011	Short (e.g. 3–4 years) or long-term cycle rotations (e.g. 15–20 years, if tree regeneration is also to be favoured) grazing-excluded patches can be established	They could act as islands of propagule sources of plant and invertebrates species differing from those species occurring in the grazed matrix
Beetle decline	Ecological functions and services; Lobo et al. 2001; Nichols et al. 2008	Threats to beetle populations	There is a beetle decline in the Mediterranean, since the extensive livestock grazing regimes are being replaced by intensive agriculture and afforestation, and ivermectin is being used in grazing animals

d) Isotopic and compositional analysis

Indicator	Environmental Pressure and Reference	Environmental Consequence	Interpretation
Carbon and Nitrogen	Grazing gradient; Bai et al. 2012	Effects of grazing on C : N : P stoichiometry and ecosystem functioning	Vary strongly with precipitation
	Nitrogen gradient; Vallano and Sparks 2013	Foliar d13C and %N fluctuations	No correlation between foliar d13C and foliar %N with increasing atmospheric NO2 concentration
	Grazing gradient; Bardgett, Wardle and Yeates 1998; Hamilton and Frank 2001; Ritchie, Tilman and Knops 1998; Bardgett and Wardle 2003; Bai et al. 2012	Plant and soil stoichiometric responses to grazing	Stimulatory feedbacks between C-rich root exudates, microbial activity, and plant available soil nutrients; and shifts in plant species and functional group composition
	Grazing gradient; Bai et al. 2012	Root exudation of C-rich substances stimulated, elevated N mineralization, enhanced plant N content and reduction in C : N	Grazing under fertile conditions plays a role in element composition
	Grazing gradient; Bai et al. 2012	The C pools of all plant compartments decline significantly; the N pools in above-ground biomass and litter are also reduced by grazing	Grazing changes the contents and stoichiometry of the plant community through a cascade of plant–soil feedback
	Grazing gradient; Bai et al. 2012	Decrease in C : N ratios	Grazing in the meadow and typical steppes
	Grazing gradient; Bai et al. 2012	In the typical steppe, C : N ratio changed little in the top 20-cm soil layer and decreased in soil depths of 20–60 and 60–100 cm	C:N ratio may not vary much with grazing, depending on the community type
	Grazing gradient; Bai et al. 2012	Reduction in C and N pools in above-ground biomass and litter at the grazed sites, grazing increased the N contents (%) of both above-ground biomass and litter in meadow and typical steppes; increases in N content in the typical steppe	Justified by a substantial decrease in standing biomass and litter
Carbon	Elemental variability; Virgona and Farquhar1996 (Condon et al. 1987, Ehleringer1990, Ehleringer et al. 1990, Virgona et al. 1990, Meinzer et al. 1992) (Sparks and Ehleringer 1997, Schulze et al. 1998) (Vitousek et al. 1990, Hultine and Marshall 2000, Williams and Ehleringer 2000 (DeLucia et al. 1988, Schuster et al. 1992b (Ehleringer 1993b, Poorter and Farquhar 1994). Dawson et al 2002	C isotopes can be used to assess traits that co-vary with gas exchange, C gain, and water relations	Including water use efficiency (WUE), photosynthetic capacity, stomatal conductance, leaf nitrogen content, leaf mass per area, longevity, and relative growth rate

	Elemental variability; Dawson et al 2002	C isotopes can be used as an ecological index of plant function	There is a correlation between habitat quality and the biochemical discrimination against $^{13}C0_2$ during gas exchange
	Elemental variability; Ehleringer and Cooper 1988; Ehleringer 1993a, 1993b; Stewart et al. 1995; Korol et al. 1999; Madhavan et al. 1991; Comstock and Ehleringer 1992; Panek and Waring 1997; Ehleringer et al. 1986; Zimmerman and Ehleringer 1990; Welker et al. 1993; Panek and Waring 1995; Condon et al. 1992; Hogberg et al. 1993; Guehl et al. 1995; Bowman et al. 1989; Sandquist and Ehleringer 1995; Poss et al. 2000; Bettarini et al. 1995; Ehleringer and Cerling 1995; Williams et al. 2001; Dawson et al 2002	Carbon discrimination variation	Soil moisture , low humidity , irradiance , temperature, nitrogen availability, salinity , and atmospheric CO2 concentration
	Elemental variability; Geber and Dawson 1990; Vitousek et al. 1990; Hanba et al. 1999; Hultine and Marshall 2000; Hultine and Marshall 2000; Waring and Silvester 1994; Panek and Waring 1995; Panek 1996; Walcroftet al. 1996; Warren and Adams 2000; Yoder et al. 1994; Martinelli et al. 1998; Dawson et al 2002	Carbon variation in Δ	Affected by leaf size and thickness, stomatal density , branch length, and canopy height
	Elemental variability; Farquhar and Richards 1984; Henderson et al. 1998; Virgona and Farquhar 1996; Condon et al. 1987; Ehleringer 1990; Ehleringer et al. 1990; Virgona et al. 1990; Meinzer et al. 1992; Sparks and Ehleringer 1997; Schulze et al. 1998; Vitousek et al. 1990; Hultine and Marshall 2000; Williams and Ehleringer 2000; DeLucia et al. 1988; Schuster et al. 1992b; Ehleringer 1993b; Poorter and Farquhar 1994; Dawson et al 2002	Because of the integrative response of Δ to multiple eco-physiological constraints through time, C isotopes can be used to assess traits that co-vary with gas exchange, C gain, and water relations	This includes water use efficiency (WUE) , photosynthetic capacity, stomatal conductance, leaf nitrogen content, leaf mass per area , longevity, and relative growth rate
	Grazing gradient; Bardgett, Wardle and Yeates 1998; Hamilton and Frank 2001; Bai et al. 2012	Grazing often increases C-rich root exudates that stimulate microbial activity and turnover	Increase in soil nutrients available to plants
Nitrogen	Grazing gradient; Bai et al. 2012	Accelerated N cycling	N limitation reduction, N and P colimitation intensification
	Nitrogen gradient; Amundson et al. 2003; Perakis et al 2011	Isotopic composition of N inputs and losses	Natural abundance d15N isotopes provide an index of the openness of ecosystem N cycling ecosystem d15N

	Nitrogen gradient; Högberg 1997; Houlton and Bai 2009; Perakis et al 2011	Foliar and soil d15N increase	With soil N availability due to losses of isotopically depleted nitrate and trace N gases that cause d15N enrichment of soil
	Nitrogen gradient; Perakis et al 2011	Influence foliar d15N	Shifts in reliance on different soil N forms
	Foliar Nitrogen uptake; Vitousek et al. 1997; Bobbink et al. 2010; Vallano and Sparks 2013	Changes in ecosystem structure, function, and composition	Atmospheric N levels and the resulting N that is subsequently deposited to plants and soil are often associated with dramatic
	Foliar Nitrogen uptake; Handley and Raven 1992; Robinson 2001; Vallano and Sparks 2013	Stable nitrogen isotope ratios (15N/14N)	Useful for understanding spatial patterns of N cycling and identifying uptake pathways of N sources into plant metabolism because they are integrators of biological processes at the air–plant–soil continuum
	Foliar Nitrogen uptake; Vallano and Sparks 2013	Foliar d15N values that reflect the d15N of distinct N sources	May provide information regarding the causal relationships between plant uptake, assimilation, and allocation of N from natural and human-derived N sources
	Foliar Nitrogen uptake; Vallano and Sparks 2013	Foliar d15N control	By soil d15N, foliar N uptake, and mycorrhizae
	Foliar Nitrogen uptake; Robinson 2001; Kahmen et al. 2008; Vallano and Sparks 2013	Soil d15N is the primary factor influencing foliar d15N in most plant species	Foliar d15N was depleted relative to surface soil d15N, likely resulting from mineral soil N uptake that is depleted in 15N compared to the sampled soil surface N or soil organic N
	Foliar Nitrogen uptake; Gebauer and Schulze 1991;Högberg 1997; Emmett et al. 1998; Pardo et al. 2002; Templer et al. 2007; Vallano and Sparks 2013	Decreasing foliar d15N in relation to soil d15N across species may be potentially linked to lower nitrification rates in the soil N pool	Correlations between foliar d15N and relative rates of soil N cycling in temperate forests
	Elemental variability; Dawson et al 2002	Influences on plant δ15N	The presence of multiple N-sources with distinct isotopic values, mycorrhizal associations, temporal and spatial variation in N availability, and changes in plant demand
	Elemental variability; Handley et al. 1994, 1997; Dawson et al 2002	N isotopic fractionation	May be induced by very high external NO_3 concentrations, osmotic stress, or drought
Elemental discrimination	Elemental variability; Pataki et al., in press; Ometto et al., personal communication; Bowling et al. 2002; Fessenden and Ehleringer 2002; Buchmann et al. 1997; Dawson et al 2002	Ecosystem Δ is known to exhibit a high degree of variability	With factors like precipitation, water availability, vapor pressure deficit, stand age, and species composition as the major drivers
Nutrient cycling	Grazing gradient; Ritchie, Tilman and Knops 1998; Bai et al. 2012	Grazing decelerated nutrient cycling	Inhibit the growth of palatable and nutrient-rich species with high litter quality