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TESE ELABORADA PARA OBTENÇÃO DO GRAU DE DOUTOR EM ENGENHARIA FLORESTAL E DOS RECURSOS NATURAIS

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Á Lali e á Prune, aos meus pais.

Tese de doutoramento

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Titulo: **Efeito da exclusão de pastoreio e da invasão por arbustos na ecologia do Montado**

Tese apresentada ao Instituto Superior de Agronomia da Universidade de Lisboa para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Engenharia Florestal e dos Recursos Naturais, realizada sob a orientação científica da Doutora **Maria da Conceição Brálio de Brito Caldeira**, Professor auxiliar do Departamento de Recursos Naturais, Ambiente e Território (DRAT) e investigadora do Centro de Estudos Florestais (CEF) do Instituto Superior de Agronomia, do Professor **João Manuel Dias dos Santos Pereira**, Professor Catedrático Emérito do DRAT e investigador do CEF, do Doutor **Miguel Nuno Sacramento Monteiro Bugalho**, investigador principal FCT do Centro de Ecologia Aplicada “Prof Baeta Neves” (CEABN-InBIO) do Instituto Superior de Agronomia da Universidade de Lisboa.

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Effects of grazing exclusion and shrub encroachment on the ecosystem ecology of evergreen oak woodland

PhD in Forestry Engineering and Natural Resources.

Abstract

Shrub encroachment, an increase in density and cover of shrub plant communities, is affecting biodiversity and functioning of ecosystems around the world. Through feeding and trampling ungulates affect the structure and species composition of plant communities, including shrublands, and are key drivers in shrub encroachment processes. This thesis, conducted within a long-term browsing exclosure experiment, investigates the effects of ungulates (red deer *Cervus elaphus* and fallow deer *Dama dama*) on the encroachment of *C. ladanifer*, a dominant Mediterranean shrub, into a mixed cork (*Quercus suber*) and holm (*Quercus rotundifolia*) evergreen oak woodland in Southern Portugal. Five paired fenced (ungulate-excluded) and unfenced (ungulate-allowed) plots of 25 m x 25 m were established in the study area in July 2001. Fenced plots had a 2.20 m height fence to exclude deer occurring in the site. Data on *C. ladanifer* reproduction structures (buds, fruits and flowers), soil seed bank, population density and biomass, were collected in fenced and open plots in 2007, 2013 and 2015. Ungulates consumed buds, flowers and fruits and decreased the soil seed bank of *C. ladanifer*. Plant reproductive costs (loss of fruits) were higher than potential benefits (seed dissemination) and the population density and biomass of *C. ladanifer*, decreased in the open plots by the end of the experiment. Ungulates decreased above-ground carbon (C) storage but also the amount of fine fuel loads reducing fire hazard and the probability of crown fires, and consequent adult oak mortality, in open plots. Therefore ungulate may potential benefit long-term ecosystem C storage. A drought year during data monitoring allowed quantifying jointly effects of ungulate browsing and drought on *C. ladanifer* mortality which increased in open plots. Changes in live:dead plant biomass affected fire hazard. Results show that ungulate ecology needs to be considered when investigating shrub encroachment processes.

Keywords: Shrub encroachment, Plant-animal interactions, Seed predation, Fire behavior, Drought

Efeito da exclusão de pastoreio e da invasão por arbustos na ecologia do Montado

Doutoramento em Engenharia Florestal e dos Recursos Naturais

Resumo

A expansão dos matagais pode alterar a biodiversidade e o funcionamento dos ecossistemas. Pela alimentação e pisoteio, os ungulados alteram a estrutura e composição das comunidades vegetais, podendo afectar a expansão dos matagais. Esta tese investiga o efeito dos ungulados silvestres (veado, *Cervus elaphus* e gamo, *Dama dama*) na dinâmica populacional e expansão da esteva (*Cistus ladanifer*) num montado misto de sobreiro (*Quercus suber*) e azinheira (*Quercus rotundifolia*) no sul do Portugal. Em Julho de 2001 instalaram-se cinco blocos de parcelas emparelhadas de 25m x 25m: uma parcela vedada (rede caça maior 2.20m altura), para exclusão de pastoreio, e uma parcela adjacente, aberta. Em 2007, 2013 e 2015 mediu-se o número de flores, gomos e frutos de esteva e se o banco de sementes no solo, densidade populacional e estrutura e biomassa do esteval, nas parcelas vedadas e abertas. Verificou-se que os cervídeos consumiram flores, gomos e frutos de esteva tendo reduzido o banco de sementes de esteva no solo. Os custos de reprodução para a esteva (ex: perda de frutos) foram superiores benefícios (ex: disseminação de sementes) e implicaram uma redução da densidade populacional e biomassa da esteva nas parcelas abertas. Os cervídeos reduziram o armazenamento de carbono na vegetação, mas também a carga de combustível fino, e a probabilidade de ocorrência e a severidade de incêndios. Nas parcelas abertas, a probabilidade de fogo de copa foi menor, implicando uma menor mortalidade das árvores em caso de incêndio. Os cervídeos podem assim favorecer o armazenamento de carbono a longo prazo (carbono arbóreo). O ano de seca extrema de 2011-2012 permitiu ainda avaliar os efeitos combinados do pastoreio e seca na mortalidade da esteva que aumentou drasticamente nas parcelas abertas implicando também aumento do risco de incêndio. Os resultados evidenciaram a importância dos ungulados nos processos de expansão de matagais.

Palavras-chave: Invasão por arbustos, Interacções plantas-animais, Predação de sementes, Comportamento de fogo, Seca

Efeito da exclusão de pastoreio e da invasão por arbustos na ecologia do Montado

Resumo alargado

A expansão dos matagais, em parte devido a alterações do uso da terra (e.g. abandono agrícola) e factores como as alterações climáticas, é um fenómeno global que está a afectar a biodiversidade e funcionamento dos ecossistemas. O pastoreio, por herbívoros domésticos ou silvestres, pode alterar a estrutura, produtividade e composição florística de comunidades vegetais como os matagais. O pastoreio pode por isso afectar a dinâmica da expansão dos matagais. Nesta tese investigaram-se os efeitos de ungulados silvestres (veado *Cervus elaphus* e gamo *Dama dama*) na dinâmica da expansão de matos de esteva *Cistus ladanifer* em montado de sobreiro (*Quercus suber*) e azinheira (*Q. rotundifolia*) numa área de estudo no Alto Alentejo em que ocorrem populações daqueles animais. Os dados foram recolhidos no âmbito de uma experiência de exclusão de pastoreio, de longo prazo (mais de 15 anos) que se mantém em curso. Em Julho de 2001 instalaram-se 5 blocos de parcelas emparelhada de 25 m x 25 m cada uma. Cada bloco é constituído por uma parcela vedada com rede de caça maior de 2.20 m de altura, para exclusão dos cervídeos (veado e gamo) e uma parcela controlo, aberta, adjacente àquela. Quando estas parcelas foram instaladas não existia esteva no terreno, mas esta espécie arbustiva começou a colonizar as parcelas, vedadas e abertas, a partir 2003. Para avaliar os efeitos dos cervídeos na dinâmica do esteval fizeram-se várias medições em indivíduos de esteva em 2007, 2013 e 2015.

Assim, para avaliar o efeito daqueles animais no ciclo reprodutivo da esteva monitorizou-se, em indivíduos amostrados aleatoriamente em parcelas abertas e vedadas, o número de gomos, flores e frutos de esteva. Verificou-se que os cervídeos consumiram estruturas reprodutivas daquele arbusto ao longo de todo o ano e também que o consumo de gomos e flores foi superior ao de cápsulas, sugerindo que potenciais efeitos positivos dos cervídeos (ex: disseminação de sementes) no ciclo reprodutivo da esteva são suplantados pelos efeitos negativos (ex: menor formação de frutos) (capítulos 1 e 2). Verificou-se também que existem outros predadores da esteva, nomeadamente invertebrados e pequenos roedores, cuja actividade é indirectamente influenciada pelos cervídeos (ex: diminuição disponibilidade de frutos); o pastoreio por cervídeos resultou num aumento de

75% da predação por invertebrados. Este estudo e resultados são apresentados no Capítulo 2 desta tese.

Em 2007 e 2013 avaliou-se a densidade populacional (i.e. número de estevas em cada parcela e tratamento) do esteval e o fitovolume em indivíduos de esteva aleatórios nas parcelas vedadas e abertas, tendo também sido quantificado o banco de sementes de esteva do solo em 2010. Verificou-se que os ungulados diminuíram 33.6 vezes o fitovolume entre 2007 e 2013. O banco de sementes de esteva no solo, nas parcelas abertas, diminui também cerca de 2.6 vezes relativamente ao das parcelas vedadas (Após nove anos de exclusão dos cervídeos). Verificou-se que a densidade populacional de estevas diminui cerca de 3 vezes, entre 2007 e 2013 nas parcelas abertas (ao contrário das parcelas vedadas em que a densidade populacional aumentou de cerca de 2.6 vezes). Em 2013, a densidade populacional era superior cerca de 10 vezes nas parcelas vedadas. Estes resultados corroboram a hipótese de que os cervídeos afectaram negativamente o ciclo reprodutivo da esteva provocando a diminuição da densidade populacional, sugerindo que estes animais podem controlar a expansão da esteva. Este estudo corresponde ao capítulo 3 da tese.

Em 2007 e 2015 avaliou-se também a biomassa em indivíduos de esteva aleatórios nas parcelas vedadas e abertas. A quantificação da biomassa foi feita relacionando o fitovolume em indivíduos de esteva aleatórios com a sua biomassa total, estimada por amostragem destrutiva. Verificou-se que a biomassa dos indivíduos diminuiu drasticamente nas parcelas abertas entre os anos de amostragem (2007 e 2015). Pelo contrário, nas parcelas vedadas a a biomassa das estevas aumentou significativamente durante o período da amostragem. Estes resultados corroboram os efeitos negativos dos cervídeos.

Os matagais podem gerar diferentes serviços dos ecossistemas (isto é, benefícios que a humanidade retira dos ecossistemas). O armazenamento de carbono e fornecimento de alimento para ruminantes domésticos ou silvestres são dois exemplos. Podem também, nomeadamente em sistemas de clima Mediterrânico, caracterizado por verão quente e seco, gerar perigo de incêndio elevado. Os ungulados silvestres, através da alimentação, podem reduzir a biomassa aérea e o armazenamento de carbono. No entanto, ao reduzir a biomassa arbustiva e o combustível fino, aqueles animais podem reduzir também o perigo de incêndio. Neste estudo quantificou-se o carbono acima do solo disponível nas parcelas vedadas e abertas, e caracterizou-se o combustível arbustivo. Através de modelação quantificou-se também o perigo de incêndio nas parcelas vedadas e abertas. Verificou-se que os cervídeos diminuíram significativamente o carbono acima do solo (cerca de 6 vezes em media), mas também o perigo e severidade de incêndio. Mais ainda, evidenciou-se que os cervídeos diminuíram a probabilidade de ocorrência de fogo de copa e, como tal, a mortalidade das árvores. Estes resultados sugerem que ao favorecer a sobrevivência de árvores adultas (principais reservatórios de carbono dos ecossistemas) em caso de incêndio, os cervídeos poderão estar a contribuir para o armazenamento de carbono a longo termo dos ecossistemas. Este estudo corresponde ao Capítulo 4 da tese.

Os cenários de alterações climáticas prevêem maior frequência e intensidade de eventos extremos como por exemplo secas severas. Em 2012 ocorreu um ano de seca extrema, em Portugal e em toda a Europa. Este evento gerou a oportunidade de testar como podem seca e pastoreio por cervídeos afectar conjuntamente a mortalidade da esteva. De facto, a contagem em 2013 e 2015 de plantas de esteva, mortas e vivas, evidenciou um número de estevas mortas significativamente superior nas parcelas abertas. Estes resultados sugerem que pastoreio e seca aumentaram a mortalidade de estevas. Mais ainda, o aumento da proporção de material vegetal seco nas parcelas gerou, por modelação, maior perigo de incêndio, com um efeito mitigador do pastoreio. Estes resultados sugerem que as consequências de eventos extremos como a seca e o fogo são afectados pelo pastoreio tornando estas relações complexas. Este estudo corresponde ao Capítulo 5 da tese.

Os resultados globais da tese sugerem ser crucial considerar os efeitos de animais como os ungulados silvestres na dinâmica do esteval, e provavelmente outros tipos de matagais, podendo, dependendo da carga e tipo de animal, controlar a expansão dos matos. Ao alterarem a estrutura dos matagais e a disponibilidade de combustível fino, aqueles animais podem reduzir significativamente o perigo de incêndio e, no longo prazo, contribuir para o armazenamento de carbono. Finalmente, estas relações deverão ser consideradas em contexto de alterações climáticas, nomeadamente ocorrência de eventos extremos como as secas.

Contents

Chapter 1

Chapter 2

2 **Effects of multispecies interactions on the reproductive success of *C. ladanifer***

Chapter 3

3 Long-term effects of deer browsing on seed bank and population dynamics of *C. ladanifer*

Chapter 4

4 Long-term effects of deer browsing on the trade-off between above-ground carbon stock and fire hazard

Chapter 5

5 Combined effects of deer browsing and drought on *C. ladanifer* mortality and fire hazard

Chapter 6

CHAPTER 1

GENERAL INTRODUCTION

1 General Introduction

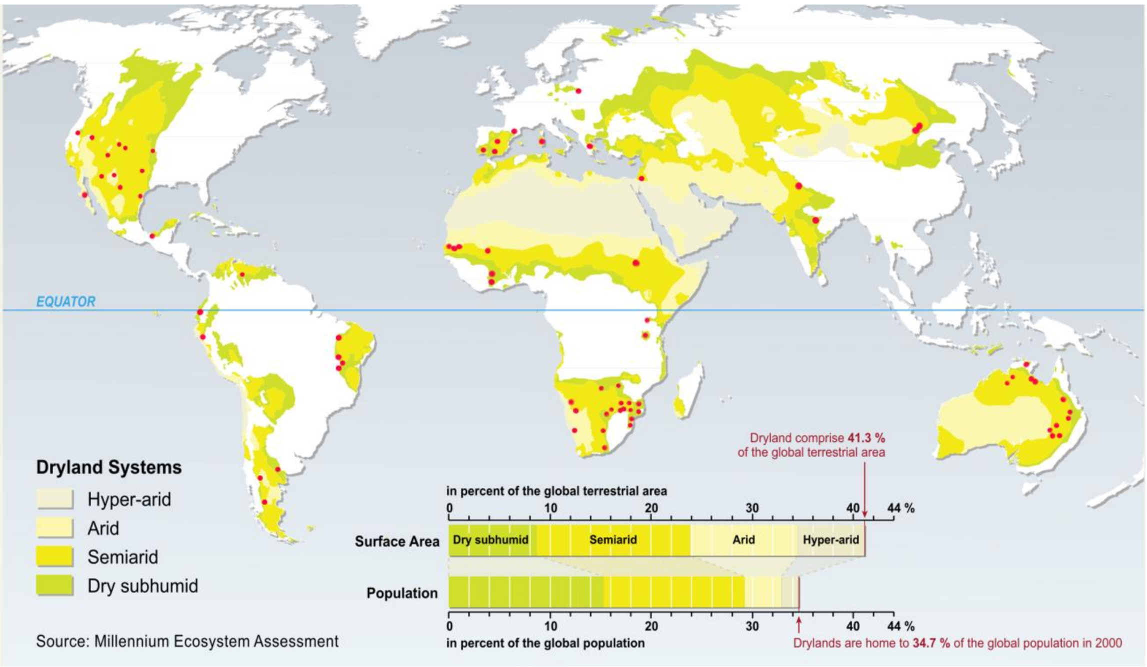
1.1 Shrub encroachment, what do we know?

Woody encroachment, i.e., the increase in density, cover and biomass of woody plants is a global phenomenon expected to increase under climate change scenarios (Blois et al. 2013). Causes of encroachment are complex and difficult to relate to a single factor. Climate and land use changes, elevated levels of CO2, nitrogen deposition or combinations of these factors are possible drivers of encroachment (Van Auken 2000, 2009, Eldridge et al. 2011, D'Odorico et al. 2012, O'Connor et al. 2014, Sala and Maestre 2014, Archer et al. 2017).

The magnitude of shrub encroachment varies with spatio-temporal scales (Barger et al. 2011, Stevens et al. 2017) depending on climate, geomorphology, topography and disturbance regimes (e.g. grazing and fire regimes) (Archer et al. 2017). For example, mean annual precipitation (MAP) is a key regulator of ecosystem productivity therefore affecting magnitude of encroachment (Knapp et al. 2008). Indeed plant productivity was shown to decrease below a threshold of 336 mm of MAP in North American ecosystems (Barger et al. 2011), while in African savannas, maximum shrub cover was found at a 650 mm threshold (Sankaran et al. 2005). Rate of shrub encroachment is also generally higher at the early stages of encroachment, declining or fluctuating afterwards (Fensham et al. 2005, Browning et al. 2008) until maximum cover is reached.

Shrub encroachment has important implications for ecosystem functioning and services (i.e. the benefits ecosystems generate to humankind, MEA 2005) including carbon sequestration, water budget or soil fertility (Van Auken 2009, Eldridge et al. 2011, Sala and Maestre 2014, Soliveres et al. 2014). These effects may be contrasting and are often site specific depending not only on climate and soil but on specific shrub traits as well (Schuman et al. 2002, Barger et al. 2011, Eldridge et al. 2011). Shrub encroachment has been associated with reduction of grassland productivity and plant species richness, with an overall loss of biodiversity, and often with expansion of unpalatable shrub species (Archer 1996, Scholes and Archer 1997, Knapp 2008, Knapp et al. 2008, Bugalho et al. 2011, Ratajczak et al. 2012). Shrub encroachment, however, may benefit the soil, through reduction of soil surface temperature, and increased water infiltration and content (Moro et al. 1997). Shrub encroachment may also increase soil nutrient turnover (e.g. carbon, nitrogen, phosphorus) (Moreno and Obrador 2007, Simões et al. 2009, Gomez-Rey et al. 2013, Blaser et al. 2014). All these effects vary with aridity gradient and may reverse in more arid systems (Soliveres et al. 2014). Shrub encroached ecosystems are highly dynamic but changes in relative abundances of grass and woody plants and consequences for ecosystem functioning may occur at decadal time-scales (Archer 1996, House et al. 2003). Hence, long-term studies are needed to assess the effects of shrub encroachment on ecosystem functioning and services.

Changes in vegetation structure due to encroachment are especially marked in drylands such as arid and semiarid areas or some Mediterranean regions (fig. 1). Such changes have often been linked to desertification (e.g. soil erosion and soil fertility loss) (Schlesinger et al. 1999, MEA 2005, Li et al. 2008, Soliveres et al. 2014). Drylands cover approximately 41% of the terrestrial earth surface, host more than 30% of the world's population and support most of the world livestock production as well as large mammal populations (White et al. 2000, MEA 2005, Safriel and Adeel 2005) (Fig. 1). Woody encroachment into grassland in these regions, with consequent reduction of grassland productivity, reinforces the need to enhance use of shrub species by livestock and other ruminants (Estell et al. 2012). It is therefore important to understand how shrub species interact with animal use (e.g. domesticated or wild livestock) and consequently affect ecosystem functioning.



Source: Millennium Ecosystem Assessment

Figure 1: Global dryland distribution divided into subtype. Red points denote worldwide locations of shrub encroachment into grasslands and savannas where encroachment occurred during the last decades and investigated research into this topic (sources: Map from MEA 2005; research papers for pointed locations: Roques et al. 2001, Bajocco and Ricotta 2007, Castro and Freitas 2009, Maestre et al. 2009, Magda et al. 2009, Van Auken 2009, Montané et al. 2010, Barger et al. 2011, Cesa and Paruelo 2011, Gomez-Rey et al. 2013, DeMalach et al. 2014, González-Roglich et al. 2014, Lovreglio et al. 2014, Saintilan and Rogers 2015, Stevens et al. 2017, Xystrakis et al. 2017).

1.2 Fire, herbivory and shrub encroachment

Humans affect vegetation species composition namely through changes in fire (i.e. prescribed fire, wildfire) and grazing (i.e. overgrazing, cessation of grazing) regimes which frequently promote shrub encroachment. In many arid and semi-arid environments, prescribed fires may contribute to maintain shrub populations at low densities, by suppressing recruitment of mature shrubs and limiting their chance of expansion. Simultaneously, prescribed fire may also enhance herbaceous vegetation productivity (Lohmann et al. 2014, Livingston et al. 2016, Stevens et al. 2017). Contrastingly, fire suppression may induce shrub encroachment (Moreira 2000, D'Odorico et al. 2006, Staver et al. 2011). The “overgrazing hypothesis” states that chronic heavy grazing (i.e. grass and herb consumption) species by livestock will reduce grass biomass (both above- and belowground) and its ability to compete with shrub species by increasing resource availability for shrub recruitment (Coetzee et al. 2008). Once shrubs are established, grazing may also contribute to seed dissemination. This will mainly occur if grazing occurs during shrub fruiting stages thus promoting shrub expansion (Albert et al. 2015). Additionally, grazing may interact with wildfire. Indeed, changes in grass productivity, growth and reproduction or re-growth failure after grazing will reduce the frequency and intensity of wildfires and may favor shrub establishment and expansion once soil resources become available (Van Auken 2009, O'Connor et al. 2014). Although all these processes are likely to occur, their relative importance for ecosystem functioning remains unclear and may change with environmental conditions (Roques et al. 2001, D'Odorico et al. 2012). For example, although overgrazing is often associated with shrub encroachment, high browsing (i.e. woody plants consumption) pressure can prevent encroachment and contribute to an open ecosystem structure, through delaying of shrub growth and development into mature stages and increased shrub exposure to fire (Singer and Renkin 1995, Augustine and McNaughton 2004, Cipriotti and Aguiar 2012, Sankaran et al. 2013). Research has shown that a 10-year browsing exclusion in a semi-arid African savanna induced a 7-fold and 3-fold increase of shrub recruitment and biomass, respectively, and a 2.5-fold decrease of woody plant mortality (Sankaran et al. 2013). Browsing may also affect negatively shrub reproduction by reducing seed production, seed bank and seedling recruitment (Wambolt and Hoffman 2004, Beauchamp et al. 2013). In contrast, grazing cessation (i.e. through land abandonment) may induce shrub encroachment. Additionally, grazing may also interact with wildfire negatively influencing post-fire vegetation recovery and lessening shrub encroachment (Ramirez et al. 2012, Silva et al. 2014, Silva et al. 2015). Hence, herbivory (i.e. grazing and browsing) and fire interactions, together with climate (e.g. rainfall, drought period) and soils, (e.g. nutrient and water availability), will drive shrub encroachment (Bond 2008, Archer et al. 2017, Stevens et al. 2017) (Fig. 2).

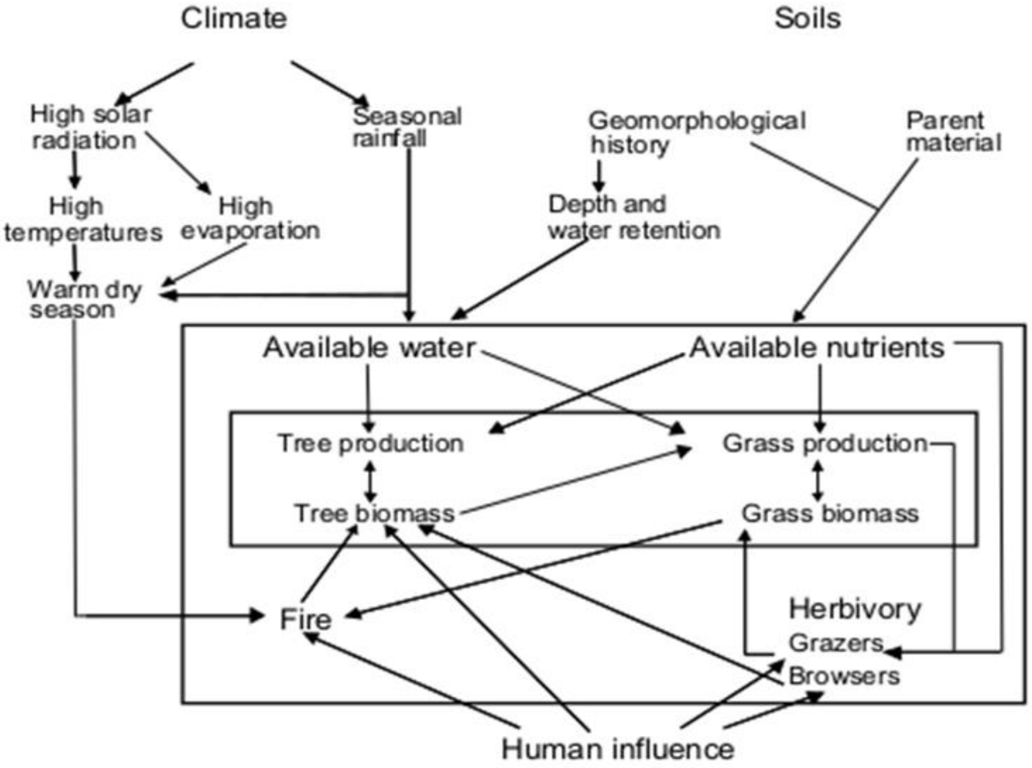


Figure 2. Key drivers for mixed tree–grass systems. Many factors interact in drylands to regulate grass and woody vegetation abundances. Determinants of vegetation structure and function (e.g. water, nutrients, fire and herbivory), which depend on outlying factors (e.g. climate and soils), affect shrub encroachment. Plant-animal interactions, namely those between woody plant species and herbivores, are a key determining factor in these systems (Scholes and Walker 1993).

1.3 Species interactions and shrub encroachment

Species interactions (e.g. competition, facilitation, predation) shape the ecology and dynamics of communities and ecosystems (Ehrlich and Raven 1964, Bascompte and Jordano 2007, Gomez et al. 2010, Kiers et al. 2010). Such interactions are context-dependent and may vary in magnitude and direction (Tylianakis et al. 2008, Chamberlain et al. 2014, Maron et al. 2014). Land-use is a crucial factor shaping species interactions and therefore the performance of individual species and populations (Vitousek et al. 1997, Sala et al. 2000, Tylianakis et al. 2008).

Mutualistic and antagonistic plant-animal interactions, for example, affect strongly the performance of individual plants and their population dynamics and, ultimately, ecosystem functioning and services (Strauss and Irwin 2004). Mutualistic interactions are frequently linked to ecosystem services such as pollination or seed dispersal (Kremen 2005). Conversely, antagonistic interactions, such has herbivory may negatively impact plants performance, by eating plant parts, reducing plant biomass, or feeding on their seeds. Plant-animal interactions are pivotal topic in the study of plant population dynamics and evolution (Thompson, 1999, 2009). Most of the studies on plant-animal interactions focus on direct, pairwise interactions. For instance, seed predation during pre-dispersal stage is commonly reported in invertebrates (Dahlgren and Ehrlén 2009, Ramírez and Traveset 2010) which may strongly affect plant recruitment and population dynamics (Kolb et al. 2007). Indeed, exclusion of invertebrate pre-dispersal seed predators reduced seed damage of Haplopappus venetus, a typical shrub of southern California, by 104% which was followed by an increase of recruitment (Louda 1983). Vertebrates, such as large mammals, birds and rodents also feed on seeds (Hulme and Benkman 2002, Fedriani and Manzaneda 2005, Gómez 2005). For example, wild ungulates such as deer consume fruits and seeds of several plant species (Suárez-Esteban et al. 2013, Perea et al. 2014) and may negatively affect reproductive biology and plant population dynamics (i.e. an antagonistic species interaction). Predation, however, depending on timing, may also benefit plant performance by promoting seed dissemination and germination (i.e. a mutualistic species interaction) (Janzen 1984, Traveset and Verdú 2001).

Plant-animal interaction outcomes generally result from multispecies interactions which, over time, may act synergistically or antagonistically, and are context dependent, being directly related to individual variation in interaction partners and contrasting strength (Strauss and Irwin 2004, Morris et al. 2007). An example, is *Lathyrus vernus* a legume occurring in Scandinavian forests which is dependent on several interactions among molluscs and insect herbivores (i.e. predators of plant buds, flowers and leaves), pre-dispersal beetle seed predators, grazers (i.e. roe deer, *Capreolus capreolus*) and pollinators (i.e. *Bombus* spp.) (Ehrlén 2002). These interactions vary intra- and inter-annually. Additionally, when several antagonists or mutualists affect the same plant species, additive, non-additive or negative combined effects may be found, because of conflicting selection pressures on the same plant trait (Strauss and Irwin 2004, Maron and Crone 2006, Morris et al. 2010). Other example of complex interactions is that of mule deer (*Odocoileus hemionus*) which, by feeding on flowering stalk of the herb *Ipomopsis* *aggregata*, decreased oviposition by the pre-dispersal seed predator fly *Hylemya* spp. by 91% in the Colorado Front Range, USA. Also, in the same area, because of deer herbivory, the presence of caterpillar feeding on *I. aggregata* flowers, fruits and seeds decreased by 48% (Juenger and Bergelson 1998). Ungulate-exclusion of Spanish ibex (*Capra pyrenaica*) in Serra Nevada (Spain), which feeds on *Erysimum mediohispanicum* flowers and green fruits, was also shown to increase seed predator abundance by 56.2%, but non-additive effects were found on predation rates (Gómez and González-Megías 2007). Complex interactions such as those presented above may act on shrub species and affect shrub encroachment.

1.4 Ecosystem services and shrub encroachment

Shrub encroachment provides a range of ecosystem services including carbon sequestration (McKinley and Blair 2008, Daryanto et al. 2013) or herbivore feeding (Bugalho and Milne 2003, Papanastasis et al. 2008, Azorit et al. 2012). Plant productivity through shrub encroachment may result in storage of above- and below-ground carbon storage. Carbon storage and sequestration however may trade-off with increased wildfire hazard (Fernandes 2009). Such trade-offs may be mediated by plant-animal interactions namely ungulate ecology.

Carbon sequestration and storage

Shrub encroachment may increase ecosystem carbon storage through net plant productivity (ANPP) (Li et al. 2016). Carbon storage resulting from ANPP has been reported in different ecosystems (Eldridge et al. 2011), including Mediterranean ecosystems (Navarro Cerrillo and Blanco Oyonarte 2006, Ruiz-Peinado et al. 2013). Shrub and woody species generally have lignin-rich tissues, which slow down decomposition rates (Melillo et al. 1982), and deeper roots systems (Jackson et al. 1996, Jobbagy and Jackson 2000) where decomposition and microbial activity are slower (Sombroek et al. 1993). Organic matter input from woody plants therefore tends to accumulate in the soil. High above- and belowground productivity and reduced decomposition rates may thus lead to increment of ecosystem carbon inputs (Liao et al. 2006, Throop and Archer 2007, Knapp et al. 2008, Zhou et al. 2017). Shrub encroached grasslands, in USA as a whole, were estimated to sequester 120 Tg C/year accounting for 30% of the total annual carbon sink (McKinley et al. 2011). Similar patterns of carbon sequestration in encroached systems were found across the world in other regions (Janssens et al. 2003, Piao et al. 2009, Eldridge et al. 2011).

Wildfire hazard

Shrub encroached systems are highly vulnerable to fire (Cardille and Ventura 2001, Yang et al. 2007, Catry et al. 2009, Rego and Silva 2014) particularly in the Mediterranean regions where encroachment is frequently driven by land abandonment (Nunes et al. 2005, Bajocco and Ricotta 2007, González and Pukkala 2007, Marques et al. 2011). The typical dense spatial structure and generally high flammability of Mediterranean shrub species make Mediterranean shrublands particularly fire-prone ecosystems. Fire propagation, for example, depends on amount of fuel, its structure and connectivity (i.e. vertical and horizontal arrangement) (De Luis et al. 2004, Fernandes 2009). The plant fuel moisture content as well as weather conditions (Dennison et al. 2003, Pellizzaro et al. 2007) determine how easily vegetation ignites. The increased frequency of severe wildfires, namely in Mediterranean regions, led to reinforcement of management measures aiming to reduce fire strength (Olmi and Joyce 2003, Costa Alcubierre et al. 2011, Fernandes et al. 2013). Using herbivores as management tools to reduce vegetation fuel loads and prevent fire hazard can be an effective management practice to reduce the probability of ignition, spread and wildfire intensity (Ingram et al. 2013). Indeed, goats have been used in Mediterranean areas to disrupt the horizontal and vertical fuel continuity, litter depth and fine fuel accumulation therefore mitigating fire hazard (Lovreglio et al. 2014). Wild ungulate herbivores can also decrease fire hazard through effects on vegetation structure (Hobbs 2006). Trade-offs among carbon storage and fire hazard in shrub encroached ecosystems, as mediated by ungulates, is a crucial point to understand the dynamics of shrub encroachment and its effects on ecosystem services.

1.5 Climate change, shrub encroachment and browsing

Climate change scenarios forecast an increase in frequency and severity of extreme events such as droughts and heat waves (IPCC 2014). Climate changes may therefore affect biodiversity (Bellard et al. 2012, Peñuelas et al. 2013) and ecosystem services (e.g. carbon sequestration, water) (Hooper et al. 2005) and ultimately human well-being (MEA 2005, Cardinale et al. 2012).

Drought, in particular, may reduce ecosystem functioning and resilience (Caldeira et al. 2015) and induce woody plant mortality (Allen et al. 2010, Camarero et al. 2015) through mechanisms such as hydraulic failure and carbon starvation (Adams et al. 2009, McDowell et al. 2011) or by increasing susceptibility to pathogens (Jactel et al. 2012, Sangüesa-Barreda et al. 2015), causing tree mortality (Bréda et al. 2006). In shrub encroached ecosystems, increased woody plant mortality due to drought may promote accumulation of dry and highly flammable fuel and increased wildfire hazard (Anderegg et al. 2013).

Drought also affects grass productivity negatively (Knapp and Smith 2001, Haddad et al. 2002), and thus may exacerbate consumption of woody plant species (i.e. browsing) by ungulate herbivores. Indeed, woody species become an important source of forage for ungulate herbivores, namely ruminants, when grass availability becomes limited (Bugalho and Milne 2003, Papanastasis et al. 2008, Azorit et al. 2012). Therefore increased browsing pressure during drought events may increase negative effects on woody plant species performance and eventually increase plant mortality (Watson et al. 1997, Read 2004). Finally, accumulation of dead woody vegetation may strongly affect fire hazard (Anderegg et al. 2013). Investigating how drought may act together with browsing in shrub encroached systems and affect fire hazard is of relevance, particularly in the Mediterranean fire prone shrublands.

1.6 Aims of the thesis

The overall aim of this thesis is to understand how ungulate browsing exclusion drives shrub encroachment and affects ecosystem structure and functioning of an evergreen cork (*Quercus suber*) and holm (*Q. rotundifolia*) oak woodland in southern Portugal. Through a long-term ungulate herbivory exclusion experiment, the thesis investigates how deer (red deer *Cervus elaphus* and fallow deer *Dama dama*) affects the structure, reproductive success and population dynamics of *Cistus ladanifer*, a semi-deciduous Mediterranean shrub species that dominates oak woodland understory in the study area. Additionally, the thesis addresses how deer may mediate ecosystem service trade-offs in shrub encroached areas, namely carbon storage and fire hazard mitigation and how these effects may be shaped by drought. In particular, a first objective addresses seed pre-dispersal and predation by deer, mice and invertebrate predators and on how such interrelationships affect the reproductive success of *C. ladanifer* (Chapter 2). A second objective investigates the long-term effects of deer browsing on *C. ladanifer* seed bank and germination capacity and ultimately population dynamics (Chapter 3). A third objective aims to understand how deer may mediates ecosystem services trade-offs in encroached systems namely above-ground carbon stocks and fire hazard (Chapter 4). Finally, a fourth objective assesses the jointly effects of browsing and drought on shrub mortality in encroached areas and how such effects affect fire hazard (Chapter 5).

The following diagram (Fig. 3) provides a conceptual framework for this thesis summarizing the four specific objectives of the work:

I. Assessing the effects of multispecies interactions on the reproductive success of *C. ladanifer*;

II. Investigating long-term effects of deer browsing on seed bank and population dynamics of *C. ladanifer*;

III. Understanding how deer browsing may mediate trade-offs between above-ground carbon stocks and fire hazard;

IV. Evaluating how deer browsing, jointly with drought, drives *C. ladanifer* mortality affecting fuel load characteristics and fire hazard in an encroached system.

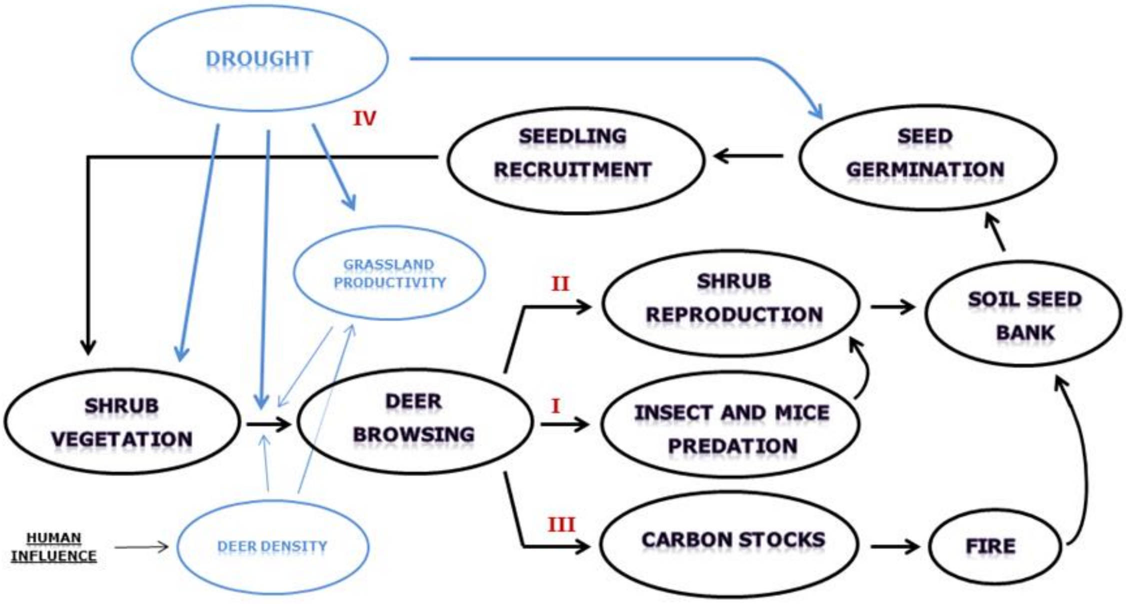


Figure 3. Conceptual framework diagram illustrating thesis objectives (I, II, III and IV). Black arrows represent stages within deer-*C.ladanifer* interactions that affect shrub population, other herbivore species (mice and invertebrates) and carbon stocks and fire hazard. Blue arrows represent effects of drought on investigated processes (black balloons).

1.7 Study area

The study was conducted in Tapada Real de Vila Viçosa, an enclosed estate of approximately 800 ha located in Southern Portugal (Fig. 4).

The climate is Mediterranean, characterized by hot and dry summers with rainfall occurring mainly between October and May. Mean annual precipitation is 585 mm and mean annual temperature 16°C respectively. Maximum temperature is 31°C, in July and minimum temperature is 6°C, in January. The year of 2011-2012 was extremely dry in the study area and all Southwest Europe (Trigo et al. 2013) with total annual precipitation in study area (i.e. hydrological year from October 2011 to September 2012) being 321mm, that is 55% of the long-term mean annual precipitation.

The vegetation is a typical Mediterranean evergreen oak woodland of cork oak (*Quercus suber* L.) and holm oak (*Q. ilex spp. rotundifolia*) and an understory of mainly annual grasslands interspersed with *C. ladanifer* which dominates the shrub layer. Grasslands are dominated by grasses (e.g. *Brisa maxima*, *Bromus madritensis*) with forbs (e.g. *Andryala integrifolia*, Leontodon taraxacoides) and legumes (e.g. Vicia disperma, *Ornithopus compressus*) occurring at lower proportions.

A population of red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) with a density of 0.35 and 0.1 deer per ha respectively at the beginning of the study occurs in the study area (Bugalho et al. 2011). Wild boar (*Sus scrofa*) is also present and its populations have increased recently.

Experimental design

In July 2001, 5 blocks of paired fenced (i.e. ungulate-excluded) and unfenced (i.e. ungulate-exposed) plots of 25 m × 25 m were established in homogeneous areas of grassland without shrubs as part of a long-term research project (e.g. Bugalho et al. 2011). All plots included 6 to 8 adult oaks trees and fences were 2.20 m tall to exclude deer. Each plot was further divided into 40 sub-plots of 2 m x 4 m to monitor *C. ladanifer* population which began colonizing the plots 2 years after plot establishment. These paired fenced-open plots were used to address objectives II, III and IV. For responding to objective I, the paired approach was not used as *C. ladanifer* population in ungulate-exposed plots was too small for monitoring the experiment. In this case other control plots, dominated by *C. ladanifer*, were selected (Fig. 4)

1.8 *Cistus ladanifer*

Cistus ladanifer, a stress tolerant woody perennial, is common to the western Mediterranean Basin, including southern Europe and North Africa (Guzmán and Vargas 2009). In the last decades, *C. ladanifer* has expanded due to land use (Costa et al. 2009) and climate changes (Acácio et al. 2009) often forming mono-specific dense layers. This species is an obligate seeder recruiting from hard seeds, encapsulated in a fruit, which form a persistent soil seed bank. Maturity of *C. ladanifer* occurs at 2 to 4 years (Talavera et al. 1993). This species is self-incompatible and mainly an entomophilous depending on generalist insect-pollinator species such as flies (e.g. Calliphoridae and Anthomyiidae), bees (Apidae) and beetles (e.g. Scarabaeidae, Malachidae) (Talavera et al. 1993, Guzmán and Vargas 2009). Flowering occurs in early spring (March-April extending until May) and fruit maturation between mid-May and beginning of July (Talavera et al. 1993). Seed release starts in mid-summer extending until early winter. Fruits are globular lignified capsules with 5–12 locules containing 300-1200 seeds of ca. 0.8 × 0.6 mm in size (Talavera et al. 1993, Delgado et al. 2008). Pre-dispersal insect predation mostly by larvae of Noctuidae (Lepidoptera) and Bruchidae (Coleoptera) may occur in this species (Serrano et al. 2001, Serrano et al. 2007). Larvae bore through the fruit wall, where they leave an open hole, and then through the septa, moving between locules to feed on seeds until completing their development (Serrano et al. 2001).

Wild ungulate such as red deer feed on *C. ladanifer* during all the plant development stages and may act both as seed predator and seed disperser (Malo and Suárez 1998, Azorit et al. 2012, Albert et al. 2015).

Germination from dormant seeds in the soil is stimulated by fire (i.e. high temperature) (Thanos et al. 1992, Ferrandis et al. 1999, Delgado et al. 2001). (Fig. 4G, H and I). *C. ladanifer* individuals are easily flammable, due to the terpene-derived resin that impregnates leaves and branches and which is easily ignited (Núnez-Regueira et al. 2005). These resins may also indirectly lessen litter decomposition and accelerate fine fuel accumulation (Horner et al. 1988).

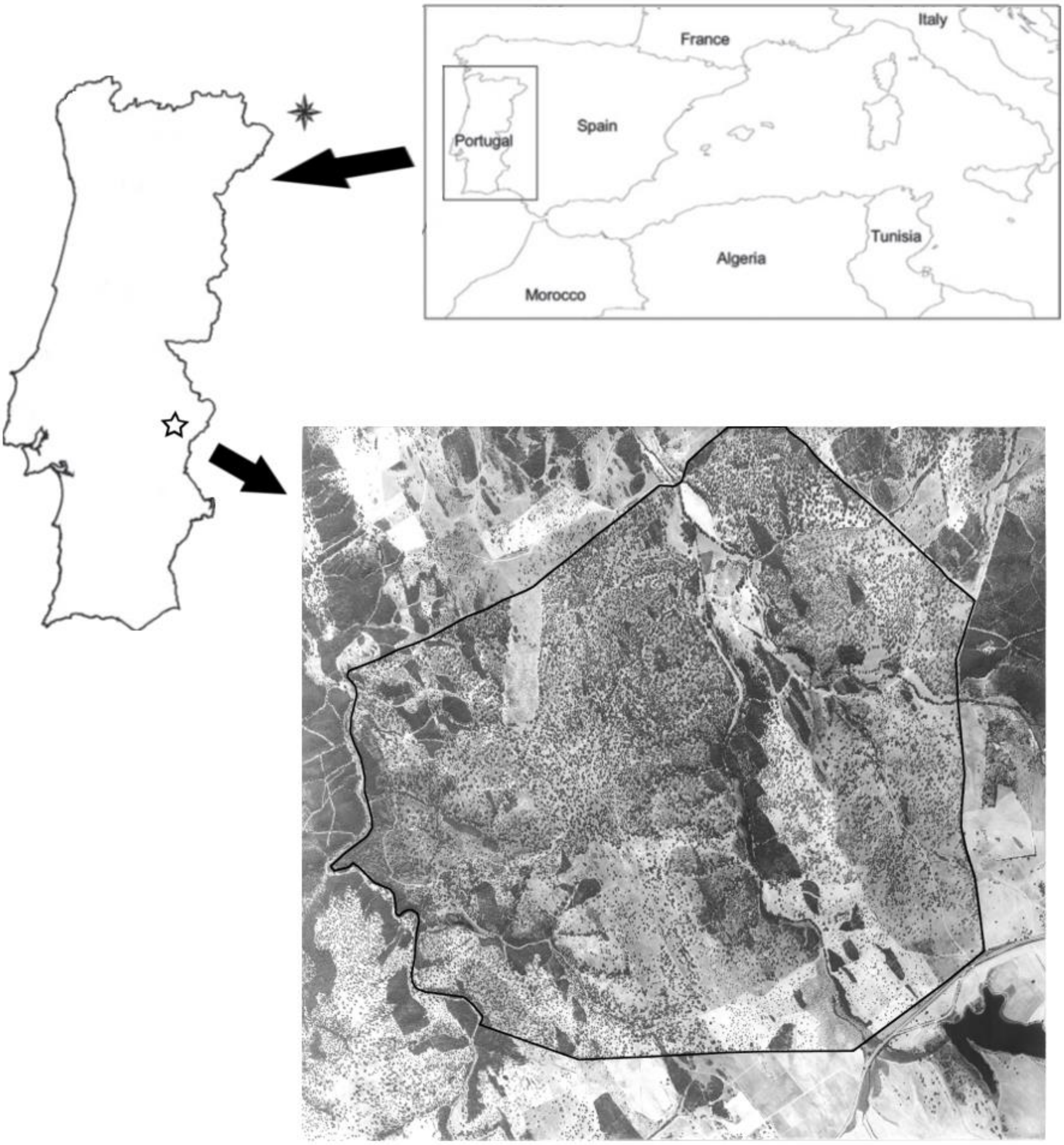


Figure 4. Portugal location within the Mediterranean basin (top right), location of the study sites in Portugal (left) and areal photograph of the estate (below right).

Figure 4. General pictures of the study area. A) Ungulate-excluded plot in 2001. B) Ungulate-exposed area in 2001 (note that in 2001, no *C. ladanifer* individuals were present within the plots). C) Ungulate-excluded plot in 2013. D) Ungulate-exposed plot in 2013 (note the paired ungulate-excluded plot in the background). E) Ungulate-exposed plot used for the objective I. F) Red deer (*Cervus elaphus*) in the study area. G) Flower and buds of *C. ladanifer*. H) Fruits of *C. ladanifer*. I) *C. ladanifer* fruit infested by coleopterans.

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CHAPTER 2

THE EFFECTS OF MULTISPECIES INTERACTIONS ON THE REPRODUCTIVE SUCCESS OF *C. LADANIFER*

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2.1 Abstract

The sum of fruit and seed predation by multiple species may strongly affect plant reproduction and population dynamics. We evaluated the combined effects of ungulates, seed-eating rodents and insect pre-dispersal seed predators on the reproductive success of the Mediterranean gum cistus shrub (*Cistus ladanifer*), over two consecutive years within a long-term ungulate-exclusion experiment. We compared fruiting success in shrubs exposed and protected from ungulates by examining fruit abortion and fruit production. We also investigated the effect of insect predation on seed production (i.e. proportion of depredated fruit and seed loss) and measured fruit weight, seed number per fruit, and seed weight of unpredated fruits. Ungulate browsing directly removed 42.3% of the plant reproductive structures, early in the reproductive season and insect predation reduced mature seeds by over 40%. Results also emphasize the additive effects of ungulate browsing on pre-dispersal insect predation and fruit abortion which increased by 74.7% and 60.9%, respectively. Rodents, which only occurred in ungulate-excluded plots, had a limited and later effect on seed production with 6% of mature fruit loss. Fruit weight, seed weight and number were higher in shrubs protected from ungulates. Our study indicated that seed predation by mice was irrelevant, but ungulate and invertebrate seed predation interacted to strongly limit the reproductive success of *C ladanifer*, potentially affecting plant population dynamics in the long-term.

Keywords: *Cistus ladanifer*; Multispecies interaction; Cascading effect; Pre-dispersal predation; Seed loss; Fruit traits; Fruit abortion; Temporal trends

2.2 Introduction

Seed predation by vertebrates and invertebrates critically limits the recruitment and dynamics of many plant populations (Fedriani and Manzaneda 2005, Mezquida and Benkman 2010, van Klinken and White 2014). Seed predation often occurs during the plant pre-dispersal phase, when seeds are removed from the mother plant, and is mostly reported in birds, rodents and invertebrates (Fedriani and Boulay 2006, Fedriani et al. 2004, Hulme and Benkman 2002). Browsing ungulates, however, often depredate plant seeds both before and after fruits and seeds have completed their development (Gómez 2003a, Gómez and Zamora 2000, Malo and Suárez 1995). In most systems, there are a diversity of fruit and seed consumers with potential to limit fruit and seed production and, ultimately, plant recruitment. This potential to impact plant population dynamics is particularly strong when multiple seed predators (e.g. vertebrates and invertebrates) act synergically over time (e.g. Louda et al. 1990, Palmisano and Fox 1997, Strauss 1991).

Ungulates (e.g. red deer, wild boar), which have recently increased worldwide because of favorable habitat changes and under-harvesting (see Côté et al. 2004), prey upon fruits and seeds of various flowering plant species (Gómez 2005, Perea et al. 2014, Suárez-Esteban et al. 2014). Pre-dispersal seed predation by ungulates may strongly affect plant recruitment and growth, phenology, number and size of flowers and seeds and even plant size, although these effects remain temporally and spatially dependent (Kolb et al. 2007). In addition, pre-dispersal seed predation by invertebrates also affects plant recruitment negatively by reducing seed sets (Dahlgren and Ehrlén 2009, Ramírez and Traveset 2010, von Zeipel et al. 2006). Thus, reproductive success of most plant species results from multiple consumers feeding on the same plant host which can lead to intriguing direct and indirect cascading effects (sensu Terborgh and Estes 2010). For instance, ungulates can affect the whole faunal assemblage, including insect populations, by reducing overall vegetation cover and altering plant community structure (Bugalho et al. 2011, Foster et al. 2014, Van Klink et al. 2015). Ungulates can directly ingest and kill significant fractions of insect seed predators (e.g. Gómez and González-Megías 2007, Or and Ward 2003). Ungulates can also reduce fruit crops to such an extent that the resulting diminished crops are unlikely to satiate invertebrate seed predators (Mezquida and Olano 2013, Peguero et al. 2014) which often deplete remaining fruits and seeds. Additionally, ungulates can affect rodent populations, by altering vegetation cover (Parsons et al. 2013) or by reducing food availability (Keesing 1998). However, few studies have documented the joint effect of different mammals and insect pre-dispersal seed predators on plant fitness (Palmisano and Fox 1997, Sallabanks and Courtney 1992, Strauss 1991). For instance, additive effects of deer herbivory and insect pre-dispersal seed predation reduced the reproduction of *Ipomopsis aggregata*, a native flowering plant from Colorado, USA (Irwin and Brody 2011). Yet, to fully understand plant reproduction and population dynamics it is essential to investigate combined effects of multispecies interactions.

We evaluated the combined effects of deer (*Cervus elaphus* and *Dama dama*), seed-eating rodents and insect pre-dispersal seed predators on the reproductive success of the Mediterranean native shrub gum cistus (*Cistus ladanifer*). We experimentally compared, over two years, early fruiting success of shrubs exposed and protected from deer herbivory and the combined effect of insect predation on seed production, within a long-term experiment (see Lecomte et al. 2016). We posed two main questions: 1) Does deer browsing affect fruit production, fruit abortion and fruit traits and 2) does deer browsing affect pre-dispersal seed predation by mice and insects? We predicted that deer would be the major cause of seed loss (Lecomte et al. 2016) but also that the combined effect of deer browsing, mice, and insect predation would further reduce fruiting success and seed production of *C. ladanifer*.

2.3 Methods

Study site

The experiment was conducted in Tapada Real de Vila Viçosa (“Tapada Grande”), a study site located in southeast Portugal (38°49’N, 07°24’W). The site is an enclosed estate, of approximately 900 ha, predominantly covered by evergreen oak woodlands. The climate is typically Mediterranean characterized by hot and dry summers and cool and wet winters. Mean annual precipitation is 585.3 mm mainly distributed between October and May. Mean annual temperature is 15.9 ºC with a maximum of 31.1 ºC (July) and a minimum of 5.8 ºC (January).

The tree layer consists predominantly of holm (*Quercus ilex* ssp. *rotundifolia* Lam) and cork oak (*Quercus suber* L.) with a mostly monospecific understorey of gum cistus (*C. ladanifer* L.), interspersed with grasslands dominated by grasses (e.g. *Brisa maxima*, *Bromus madritensis*, *Gaudinia fragilis*), with forbs (e.g. *Andryala integrifolia*, *Leontodon taraxacoides*) and legumes (e.g. *Vicia disperma*, *Ornithopus compressus*) occurring in lower proportions. The site is mainly browsed by red deer (*Cervus elaphus* L.) and fallow deer (*Dama dama* L.) with approximate population densities of 0.35 and 0.1 deer per ha, respectively (Bugalho et al. 2011). Small rodents, such as *Apodemus sylvaticus, Mus spretus* and *Rattus* spp. (Rosalino et al. 2011) are the likely local consumers of *C. ladanifer* fruits and seeds (personal observation) as they commonly occur in evergreen oak woodlands. Browsing marks left by deer (i.e. ragged edge on damaged stems) are easily distinguished from marks left by mice (i.e. sharp-angled, knife-like cut on ends of stems) (Hodge and Pepper 1998).

C. ladanifer, a Mediterranean perennial and fire-prone shrub, is an obligate seeder recruiting from hard seeds encapsulated in woody fruits and from the soil seed bank. In the last decades, *C*. *ladanifer* has expanded due to land use (Costa et al. 2009) and climate changes (Acácio et al. 2009), forming often dense and mono-specific layers. *C. ladanifer* reaches maturity at 2–4 years (Talavera et al. 1993). *C. ladanifer* is also self-incompatible and a mainly entomophilous species (Guzmán et al. 2015). Flowering occurs early in spring (March-April extending until May) and fecundation depends on generalist insect-pollinator species such as flies (e.g. Calliphoridae and Anthomyiidae), bees (Apidae) and beetles (e.g. Scarabaeidae, Malachidae) (Guzmán et al. 2015, Talavera et al. 1993). Fruit maturation occurs between mid-May and beginning of July, when fruit are mature (i.e. exposed fruit without sepals) (Talavera et al. 1993). Seed release starts in mid-summer extending until early winter. Fruits are globular lignified capsules with 5–12 locules containing 300-1200 seeds of ca. 0.8 × 0.6 mm in size (Delgado et al. 2008, Talavera et al. 1993). Pre-dispersal insect predation mostly by larvae of Noctuidae (Lepidoptera) and Bruchidae (Coleoptera) occurs in this species (Serrano et al. 2001). Larvae bore through the fruit wall, where they leave an open hole, and then through the septa, moving between locules to feed on seeds until completing their development (Serrano et al. 2001).

Experimental design and sampling

We compared seed predation in ungulate-excluded and ungulate-exposed 25 m x 25 m plots. Six plots were randomly located (250 m to 800 m apart of each other) in areas covered by *C. ladanifer*. Fenced plots (ungulate-excluded) were established in 2001 as part of a long-term project (e.g. Bugalho et al. 2011, Lecomte et al. 2016) but, due to loss of unfenced plots, we could not use the initial paired approach (ungulate-excluded x ungulate-exposed plots) in the present study; The paired exposed plots were over-browsed during the last years and only a small population of *C. ladanifer* individuals was available for monitoring (e.g. Lecomte et al. 2016). In this study, we used other ungulate-exposed areas where *C. ladanifer* density was similar to the shrub population in the ungulate-excluded plots in order to exclude shrub-density related effects (Metcalfe and Kunin 2005). *C. ladanife*r density was 0.85 ± 0.15 shrub.m-2 and 0.89 ± 0.10 shrub.m-2 (mean ± SE) in ungulate-excluded and ungulate-exposed plots, respectively (Mann Whitney U-test, U = 1208.5, *P* = 0.123).

Predation by mammals and insects was assessed monthly between February and September 2015. Seed loss due to insect predation and unpredated fruit traits were also evaluated by harvesting fruits in September 2015 and in September 2016. We haphazardly selected 20 reproductive shrubs (height: ca. 2-3 m) in each ungulate-excluded and ungulate-exposed plot and tagged 2 branches with 2 to 6 inflorescences in each shrubs (502 and 536 flower-buds in the ungulate-exposed and ungulate-excluded plots, respectively). To assess phenological growth stages, we monitored presence or absence of buds, flowers or fruits in the marked branches (see below).

Flower-bud and fruit predation by mammals

To assess the effect of fruit predation over time on *C. ladanifer* reproductive success (fruit set, seed number, seed mass), we recorded monthly (February – September 2015), in all marked branches, the number of flower-buds and fruits browsed by ungulates and rodents until seed release. The number of aborted fruits persisting on *C. ladanifer* branches, which could be clearly distinguished from normally developed fruits by their dried pedicel and ovaries, was also recorded. Early fruiting success was then calculated as the proportion of remaining developed fruits exposed to insect predation.

Fruit predation by insects

We estimated insect fruit predation monthly (February – September 2015) by recording the proportion of depredated fruits in tagged branches. Because of a high deer browsing pressure (Lecomte et al. 2016), fruits were difficult to find on the branches of shrubs occurring in ungulate-exposed plots. Thus, to estimate insect fruit predation, we used fruits from adjacent branches in the same plant or from the nearest conspecific plant containing fruits.

To estimate the proportion of damaged seeds within each insect depredated fruit, we collected a sample of 3 fruits with signs of insect predation (i.e. hole on the fruit husk) in September 2015 in the same plants (described as above). These fruits were kept at 4 ºC in the laboratory. We gathered 135 fruits per treatment. As a proxy of larval infestation we registered the number of holes per fruit (e.g. Serrano et al 2001). We also counted the total number of locules and number of invaded locules per fruit. Seed loss was estimated as the ratio of invaded locules to the total number of locules in each fruit. Finally, we recorded the number of larvae or insects present inside the fruit which were later identified. We estimated insect predation again in September 2016 by harvesting 20 fruits in 15 plants per plot and assessing the proportion of depredated fruits. All available fruits in the ungulate-exposed plots were harvested in those shrubs presenting lower number of fruits.

We again evaluated insect infestation and seed loss by collecting three fruits with signs of insect predation in each plant (total of 135 fruits per treatment).

Unpredated fruit weight, seed number and seed size

Three fully developed fruits without signs of insect predation were collected in September 2015, prior to seed release, from 15 out of 20 tagged plants (totalizing 135 fruits per treatment). Fruits were then kept at 4 ºC. Fruit weight, number of locules per fruit and total seed weight were recorded. Seed number per fruit was estimated using the software WinSeedle 2008a (Regent Instruments Inc, Canada). Over 108,000 and 95,000 seeds from ungulate-excluded and ungulate-exposed respectively, were analysed. Mean individual seed weight was estimated by dividing total seed weight by the total number of seeds per fruit. In September 2016, three unpredated fruits were again collected in the 15 plants per plot (135 fruits per treatment) to assess fruit weight, which was used as a proxy of seed number.

Statistical analysis

Proportional response variables (e.g. bud/flower and fruit predation, fruit abortion, early fruiting success, fruit predation by insects at the end of the ripening season and proportion of infested locules per fruit) were analyzed through generalized linear mixed model (GLMM), fitting binomial distribution and logit link function. A Poisson distribution and log-link function was used for seed number, and a normal distribution and identity-link function was used for fruit weight and seed weight. Ungulate exclusion was specified in the models as fixed effect and the plots and shrub individual replicates (nested within plot) were included as random factors. To compare the number of holes on the husk of fruits and the number of insect larvae found in fruit, we could not use GLMM because of the low variance in the data that lead to a final Hessian matrix that was not positive definite; thus non-parametric Mann-Whitney U-tests were used. Finally, a Median test for two independent samples was used for comparing the number of locules in the unpredated fruits, because normality and equality of variance were not assumed and a Moses test of extreme reaction (Moses 1963) was performed to determine if the variability of locules number (i.e. extreme values) was equally likely in both treatments. Pearson correlation was used to relate seed number and fruit weight of unpredated fruits. Finally when comparing 2015 and 2016 insect predation and fruit traits, we specified as fixed effects ungulate exclusion, year and their interaction while experimental plot and shrub individual (nested within plot) was included as random factor. All data were analyzed using IBM SPSS® for Windows (PASW Statistics, v. 23.0.0.0, 2015).

2.4 Results

Mammal fruit predation, fruit abortion and early fruiting success

We found an 8-fold higher fruit predation in the ungulate-exposed as compared to the ungulate-excluded plots (GLMM, $F_{1,225} = 20.478$, $P < 0.0001$) (Fig. 1A). We did not find any sign of rodent fruit predation in the ungulate-exposed plots. On the contrary, in the ungulate-excluded plots, all the marks belonged to small rodents, the only predators of the reproductive structures of *C. ladanifer*, with only less than 6% of fruits being depredated.

Fruit abortion in the ungulate-exposed plots was over 2.5-fold higher than in the ungulate-excluded plots (GLMM, $F_{1,225} = 4.675$, $P = 0.032$) (Fig. 1B). Early fruiting success, i.e. the percentage of developed fruits recorded in September 2015 which were prone to insect predation, was 171-fold higher in ungulate-excluded as compared to ungulate-exposed plots (GLMM, $F_{1,225} = 65.280$, $P < 0.0001$) (Fig. 1C).

Pre-dispersal insect fruit predation

Ungulates had a strong effect, as mean factor, on pre-dispersal insect predation (GLMM, $F_{1,198} = 13.348$, $P < 0.001$) being, on average, over 2-fold higher in ungulate-exposed than in ungulate-excluded plots. Year ($P = 0.823$) and interaction between ungulate exclusion and year ($P = 0.130$), did not have effect as mean factor, meaning a temporal consistency in the effects of ungulates (Fig. 2A). Although the number of holes of depredated fruits did not differ between treatments in the two consecutive years (Mann-Whitney U-test, $P > 0.4$), ungulates had a strong significant effect on seed loss, calculated as proportion of infested locules which was on average 1.27-fold higher in the ungulate-exposed than in ungulate-excluded plots (GLMM, $F_{1,535} = 14.646$, $P < 0.001$) (Fig. 2B). Year also had a strong significant effect on seed loss which was on average 1.42-fold higher in 2015 than in 2016 (GLMM, $F_{1,535} = 76.619$, $P < 0.001$).

Finally, there was a significant interaction between ungulate exclusion and year (GLMM, $F_{1,535} = 18.674$, $P < 0.001$) meaning that ungulate effect on seed loss was stronger in 2016 than in 2015.

More than 85 % of the sampled depredated fruits (n = 269 in 2015 and n = 270 in 2016) did not hold any adult or larval insects. Most of the insects identified in the remaining fruits were coleopterans from the Bruchidae family, and few from Erotylidae and Anobiidae families.

Temporal trends of bud-flower and fruit predation

In 2015, *C. ladanifer* flowering occurred from mid-March until the end of May. Fruiting was then monitored from the end of May until the end of July. However, by the end of June few fully developed fruits were observed (i.e. fruits without sepal). Seed release took place in August. Ungulate predation occurred mainly between February and the end of May (over 85%), with a peak in April, particularly on bud-flowers when fruits were not still mature (i.e. flowering period) (Fig. 3). Difference in predation between treatments at the end of May was strongly and significantly higher in the ungulate-exposed than in the ungulate-excluded plots (GLMM, $F_{1,225} = 20.274$, $P < 0.0001$). Rodent predation seemed to be restricted to the ungulate-excluded plots and started later by the end of May extending until September. During this period, no significant differences in fruit predation were found between ungulate-exposed and ungulate-excluded plots (GLMM, $F_{1,225} = 0.583$, $P = 0.446$). This temporal trend suggests that flower buds were the reproductive structure most often consumed by ungulates whilst rodent feed preferentially on fruits. Similarly to rodent predation, insect predation started between May and June and extended during the fruiting period reaching maximum predation in September before seed release (Fig. 3)

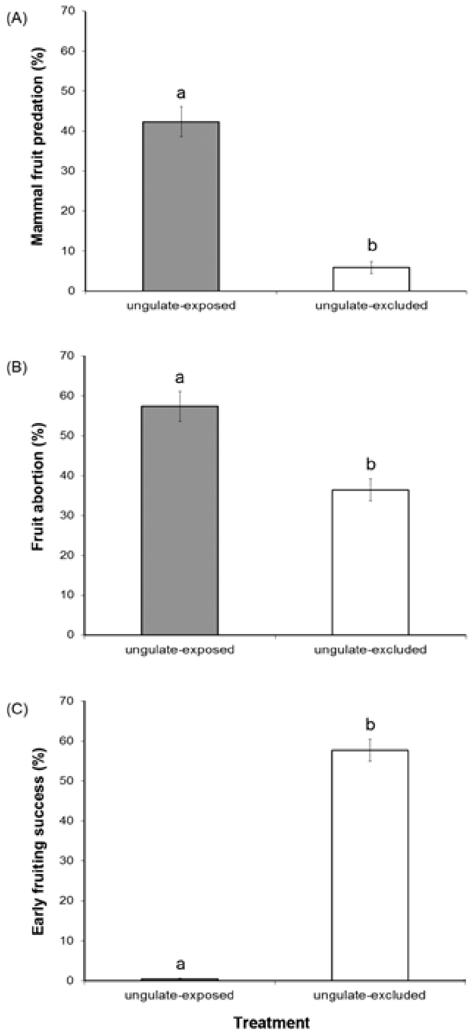


Fig. 1. (A) Total percentage of bud-flower and fruit predation by mammals in the ungulate-exposed and ungulate-excluded plots in September 2015. (B) Total fruit abortion in the ungulate-exposed and ungulate-excluded plots in September 2015 and (C) Early fruiting success (i.e. mature fruits that escape from deer predation and may be depredated by insects) in the ungulate-exposed and ungulate-excluded plots. (Mean ± SE), letters indicate significantly different values between treatments (GLMM; A and C: $P < 0.0001$ and B: $P = 0.032$)

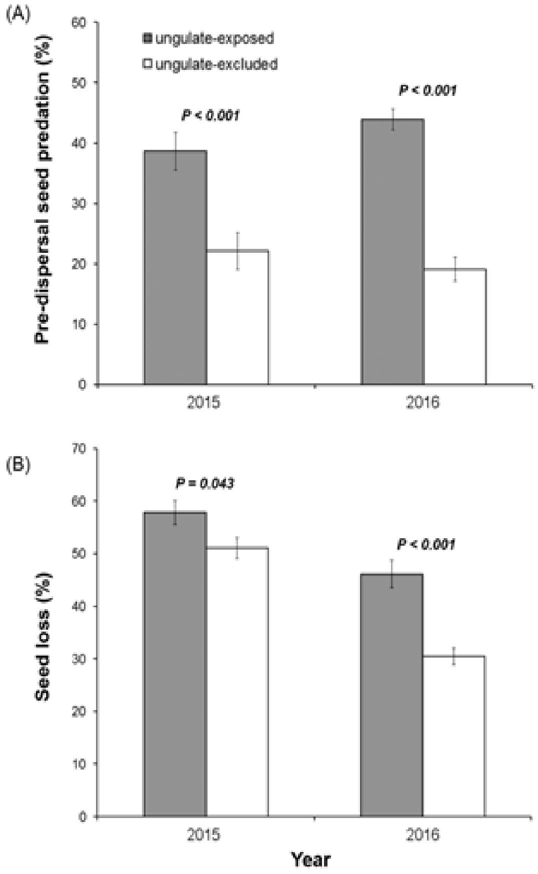


Fig. 2. (A) Total pre-dispersal seed predation by insects in the ungulate-exposed and ungulate-excluded plots in September 2015 and 2016. (B) Seed loss per collected depredated fruit by insects in the ungulate-exposed and ungulate-excluded plots. (Mean ± SE), P-values are reported for the four simple ungulate-exclusion effect.

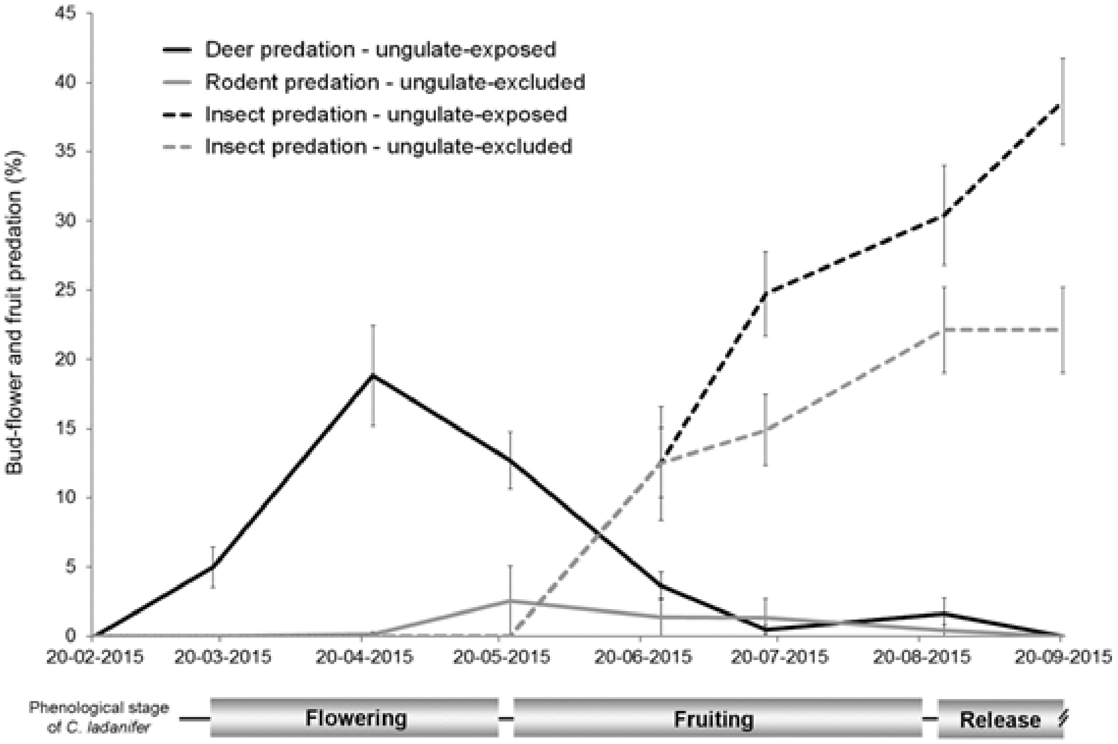
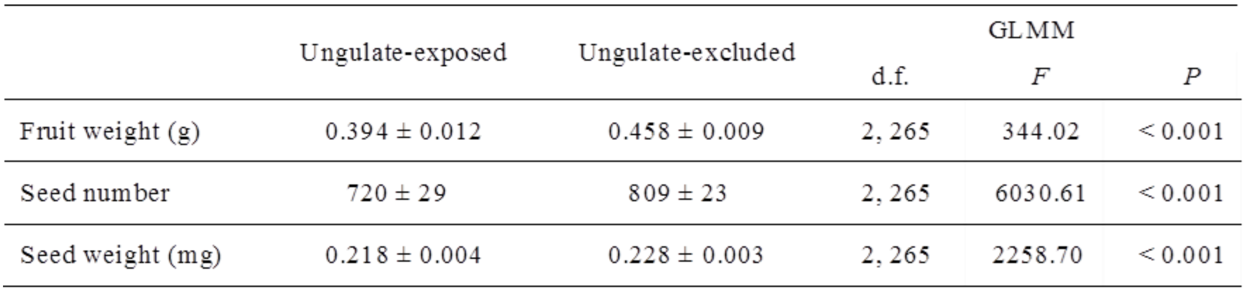


Fig. 3. Mammal predation of reproductive structures and insect pre-dispersal seed predation in the ungulate-exposed and ungulate-excluded plots during the experiment in 2015 (Mean ± SE) and observed phenological growth stage of *C. ladanifer*

Fruit weight, seed number and size of unpredated fruits

In 2015, only three out of 270 fruits lacking exterior signs of predation were infested by insects when open and thus were discarded. Fruit weight, seed number and individual seed weight were significantly higher in the ungulate-excluded plots than in the ungulate-exposed plots (Table 1). For instance, seed number and mean individual seed weight were 1.12 and 1.05-fold higher in ungulate-excluded than in ungulate-exposed plots. Seed number was positively and significantly correlated with fruit weight (Pearson correlation, $r^2 = 0.761$, $P < 0.0001$). Thus, in the ungulate-exposed plots, results showed that remaining fruits had lower fruit weight which in turn appeared to have affected seed number and individual seed weight. In 2016, fruit weight was also lower in the ungulate-exposed plots, attesting ungulate effects on seed production (GLMM, $F_{2,268} = 496.648$, $P < 0.001$). Finally, the number of locules varied between 6 and 10 in the ungulate-exposed plots and between 7 and 10 in the ungulate-excluded plots. Although mean number of locules did not differ between ungulate-exposed and ungulate-excluded plots (Grand median = 9, Z = 3.161, $P = 0.098$), the variability of locules number significantly differed between treatments (Moses test, U = 202, *P* < 0.0001), with fruits from ungulate-exposed being more likely to have fewer locules than fruits from ungulate-excluded plots.

Table 1. Fruit weight, seed number per fruit and individual seed weight in the ungulate-excluded and ungulate-exposed plots for unpredated fruit in 2015. (Mean ± SE), Statistics are reported for Generalized Linear Mixed Model



	Ungulate-exposed	Ungulate-excluded	GLMM		
			d.f.	*F*	*P*
Fruit weight (g)	0.394 ± 0.012	0.458 ± 0.009	2, 265	344.02	< 0.001
Seed number	720 ± 29	809 ± 23	2, 265	6030.61	< 0.001
Seed weight (mg)	0.218 ± 0.004	0.228 ± 0.003	2, 265	2258.70	< 0.001

2.5 Discussion

Costs inflicted by numerous antagonists may severely impact plant survival, growth and reproduction affecting the long-term population dynamics (Maron and Crone 2006). In our study, ungulate, rodent, and insect seed predation had a cumulative effect on the reproductive success of *C. ladanifer* (Irwin et al. 2011, Morris et al. 2007). This suggests that research focusing on a single seed predator guild (e.g. ungulate browsers) may not unravel overall effects of predation on target plant populations; thus, further field experiments are needed to disentangle such multispecific ecological interactions.

Mammal herbivory and reproductive success of C. ladanifer

Ungulate browsing had the largest negative effect on *C. ladanifer* reproduction. Indeed, 36.5% of the tagged buds were consumed before the end of the flowering period, corresponding to 86.4% of the total reproductive structures depredated by ungulates. Previous studies on ungulate predation also showed high flower and immature fruit consumption (Gayot et al. 2004, Gómez et al. 2000, Nóbrega Gomes et al. 2016).

However, 14% of the reproductive structures were consumed between June and August by ungulates, when seeds are fully developed and ready to be dispersed (Lecomte et al. 2016, Malo and Suárez 1998). This suggests that ungulates conferred strong costs to the seed dispersal of this shrub. Whether such costs override the benefits of mature fruit ingestion and seed dispersal deserves further investigation (Malo and Suárez 1998). These results are also consistent with our previous study on long-term interaction between *C. ladanifer* and ungulates (Lecomte et al. 2016), where the effects of mice and invertebrates were not investigated.

One unexpected result was the disclosure of a probable indirect effect of ungulates on rodents. Ungulates can negatively affect rodent abundance through the reduction of food availability (Caro 2002, Keesing 1998). As no rodent browsing marks were found in ungulate-exposed plots, it is likely that rodent predation on *C. ladanifer* reproductive structures only occurred on ungulate-excluded plots. However deer browsing may have masked rodent predation marks in the ungulate-exposed plots leading to underestimation of rodent effect. Pre-dispersal seed predation by rodents is known to occur in many herb, shrub, and tree species (Herrera 2002, Hulme and Benkman 2002). For instance, *Apodemus sylvaticus* removed up to 52% of *Helleborus foetidus* fruits before seed dispersal, in southern Spain (Fedriani et al. 2004). In our study, predation by rodents was limited to less than 6% and started in later May, which restricted possible negative effects on insect predators (Ostfeld et al. 1996). Interestingly, because rodents tend to ingest preferentially mature fruits, they could be overlooked seed dispersers of *C. ladanifer*.

Insect seed predation

Pre-dispersal seed predation by insects has been documented before for *C. ladanifer* (Narbona et al. 2010, Serrano et al. 2007). Interactions between mammal herbivores and insect seed predators, feeding on the same host plant have been shown to vary from positive (Roininen et al. 1997), neutral (Rohner and Ward 1999) to negative (Gómez et al. 2007, Herrera 1989). For instance, red deer (*Cervus elaphus*) had a positive effect on overall invertebrate abundance in a southern Spain evergreen oak woodlands (dehesa) but a neutral effect on Coleoptera and Lepidoptera (Carpio et al. 2014), which were the dominant pre-dispersal seed predators of *C. ladanifer* (Narbona et al. 2010). In our site, ungulate browsing increased insect predation by 74.7%. This positive interaction between ungulate browsing and insect predation may have resulted from a reduction of the available fruits for insects due to ungulate pressure on reproductive structures. A high insect predation of the available remaining fruits was probably due to the reduction of fruit density (Heithaus et al. 1982, Tarayre et al. 2007). While the number of holes on the husk and number of insect or larvae found on the fruit did not differ between ungulate-exposed and ungulate-excluded plots (meaning no difference in the predation pressure within the fruit) seed loss was higher in depredated fruits in ungulate-exposed plots. However, our results showed a higher variability of the number of locules within the fruits in the ungulate-exposed compared to ungulate-excluded plots. The fruits in the ungulate-exposed plots had a lower number of locules and hence fewer seeds. In addition, seed weight was also lower in the ungulate-exposed plots and larvae have to consume, in average, a higher proportion of seeds in the fruit until become satiated (Bonal et al. 2007, Crawley 2013).

Fruit abortion

Fruit set can be reduced due to pollen limitation, fruit predation, resource limitation or environmental conditions (Stephenson 1981). Pollen limitation can promote fruit abortion in *C. ladanifer* (Teixido and Valladares 2014) which may explain the high level of fruit abortion at our site. *C. ladanifer* is an early flowering species (Talavera et al. 1993) and possibly an important primary resource for generalist pollinators. This can be particularly important when shrubs form dense mono-specific stands, as in our study site, where shrub density can surpass 20,000 shrubs per ha (Caldeira et al. 2015). In our site ungulate predation occurs early in the season, reducing flower availability which may potentially decrease pollinator visits (i.e. pollen limitation) (Gómez 2003b, Sakata and Yamasaki 2015, Yoshihara et al. 2008) indirectly causing higher fruit abortion in the ungulate-exposed plots (Sabat and Ackerman 1996).

2.6 Conclusions

We showed that ungulate herbivory and insect pre-dispersal seed predation strongly reduced the availability of *C. ladanifer* seeds. Ungulates directly depredated plant reproductive structures whilst insects reduced the release of mature seeds. Seed numbers of unpredated fruits were higher in ungulate-excluded than in ungulate-exposed shrubs. We also show negative combined effects of deer browsing and insect predation on fruit and seed weight. The defoliation caused by ungulate browsing, which starts early in the season and may extend until fruiting time, may reduce fruit size (Obeso 1998, Warner and Cushman 2002).

Overall, we illustrate that ungulate and invertebrate seed predation and, to a lesser extent, seed-eating rodents interact to affect the reproductive success of *C. ladanifer* which may, in the long-term, affect its population dynamics. Further research is needed to understand the long-term effects of such multiple interactions on plant fitness and population dynamics of *C. ladanifer* and similar plant populations facing seed predation by different animal associates. Also, understanding which specific mechanisms lead to fruit abortion (i.e. pollination limitation) and the possible role of rodents and deer as seed dispersers is clearly needed.

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CHAPTER 3

LONG-TERM EFFECTS OF DEER BROWSING ON SEED BANK AND POPULATION DYNAMICS OF *C. LADANIFER*

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3.1 Abstract

Plant–animal interactions imply costs and benefits with net balance depending on interacting species and ecological context. Ungulates, in particular, confer costs (e.g., plant leaf consumption, flower bud predation) and benefits (e.g., plant overcompensation, seed dispersal) to plants. Magnitude of costs and benefits may be altered by habitat management or ecological conditions favoring high density ungulate populations. Little is known however on whether plant costs or benefits predominate over the years, or the long-term outcomes of plant-animal interactions in habitat types sustaining high density ungulate populations. We investigated how high density ungulate populations alter plant costs and benefits by quantifying ungulate long-term effects on the shrub *Cistus ladanifer* (Cistaceae) individual size, seed weight and number, seed bank, and population density, through a 12-year ungulate exclusion experiment in a Mediterranean scrubland. We monitored plant size and flower buds in plants exposed or protected from ungulates and number of developed capsules and seeds consumed (potential seed dispersal) by ungulates during three reproductive seasons. We found that ungulates negatively affected shrub size and led to a dramatically decline of shrub reproductive structures and seed production, affecting the plant reproductive cycle. Number of buds was 27 times higher and number of developed seed 5 times higher in ungulate-excluded as compared to ungulate-exposed plots. After 9 years of ungulate exclusion, the *C. ladanifer* seed bank was 2.6 times higher in ungulate-excluded plots. The population density of *C. ladanifer* was 4 times higher in ungulate-excluded plots. Our long-term experiment showed that high density ungulate populations can alter plant-animal interactions by reducing plant benefits and increasing plant costs.

Keywords: *Cistus ladanifer* L., context-dependence, deer, endozoochory, herbivory, plant-animal interaction, population density, reproductive output, seed germination, seed dispersal

3.2 Introduction

Species interactions are often described as either antagonistic or mutualistic, even though most of them correspond to a mixture of conflicting and overlapping interests, potentially being positive or negative for the participants depending on the ecological context (Holland and DeAngelis 2009, Fedriani et al. 2012, Chamberlain et al. 2014, Maron et al. 2014).

Human induced changes can alter the biotic and abiotic context and be a major driver of shifts in sign and magnitude of species interactions (Kiers et al. 2010). Hunting, for example, is known to alter plant-mammal and plant-insect interactions (Stoner et al. 2007, Harrison et al. 2013). Also, habitat management practices (e.g. limiting culling policies, food supplementation) may favor the increase of animal populations such as ungulate herbivores which can affect the ecology of ecosystems and species interactions (Rooney and Waller 2003, Côté et al. 2004). Ungulate herbivores, in particular, confer costs (e.g. plant leaf consumption, flower bud predation) (Kay 1995, Huffman and Moore 2003) and benefits (e.g. plant overcompensation, seed dispersal) (Malo and Suárez 1995, Paige 1999, Fornara and Du Toit 2007) to plants. The magnitude of such costs and benefits however may be altered by the ecological conditions namely ungulate population densities. For example, exclusion of large herbivores resulted in a shift from mutualistic to antagonist interactions in African tree-defender ants and acacias (Palmer et al. 2008). Conversely, high density ungulate populations resulting from favorable land use changes (e.g. increase of suitable woodland habitat following land abandonment) together with lack of predators and limited culling policies may affect the whole ecology of ecosystems and of species interactions (Rooney and Waller 2003, Côté et al. 2004,Bugalho et al. 2013, Frerker et al. 2014). We are far, however, from understanding how high density ungulate populations may alter the patterns, mechanisms and outcomes of plant-animal interactions (Kiers et al. 2010, Thompson 2013). One of the main obstacles to such an understanding is the lack of well-designed long-term field experiments allowing rigorous estimates of the effects of vertebrate herbivores on plants, both at the individual and the population levels. Because species interactions are critical for ecosystem functioning and ecosystem services delivery (Côté et al. 2004, Kiers et al. 2010, Traveset and Richardson 2014) further research on how human induced changes leading to high population densities of ungulates may alter plant-herbivore interactions, is clearly needed.

Ungulate herbivores consume plant leaves and often flower buds (herbivory costs) as well as fully-developed fruits comprising viable seeds (seed dispersal benefits) (Janzen 1984, Malo and Suárez 1995). The nature of such two-phase plant-ungulate interactions is expected to be mostly antagonistic if, in the long-term, herbivore populations affect negatively fruit production or exert too strong bud predation leading to too few, if any, seeds completing their development and being dispersed. For instance, deer herbivory (*Odocoileus hemionus* and *Cervus elaphus*) in a North American ponderosa pine forest reduced biomass and reproductive success of the shrub *Ceanothus fendleri* by 92% and

85%, respectively (Huffman and Moore 2003). Likewise, herbivory by a high population density of red deer in the Greater Yellowstone ecosystem, USA, practically eliminated seed production of several Rosaceae and Elaeagnaceae shrubs (Kay 1995). Conversely, different studies have shown that ungulates can act as effective seed dispersers (Malo and Suárez 1995, Iravani et al. 2011, Albert et al. 2015).

Furthermore, changes in fruit production due to leaf herbivory or direct bud or mature seed predation and potential seed dispersal, are likely to alter soil seed banks (Sternberg et al. 2003, Chaideftou et al. 2009), which are crucial determinants of the dynamics of many plant populations (Baskin and Baskin 2014). Long-term studies accounting for critical plant performance components (e.g. seed set, seed bank, seedling numbers) and changes in plant population density are thus needed for comprehensively understanding the long-term ecological effects of high density ungulate populations on the nature of plant-animal interactions.

In this study, we experimentally investigate the long-term effects of high-density populations of ungulates (red deer *Cervus elaphus* and fallow deer *Dama dama*) on plant size and several sequential reproductive components of *Cistus ladanifer* L. (Cistaceae), a seed-bank forming Mediterranean shrub, through an ungulate exclusion experiment. To identify the extent to which ungulate acted mostly as predators (antagonistic) or as potential seed dispersers (mutualistic), we estimated, separately, how long-term herbivory affected 1) plant size and subsequent flower-bud and seed production and 2) numbers of developing flowerbuds and fully developed fruit capsules and 3) seed bank in ungulate-excluded (fenced) and ungulate-exposed (unfenced) plots. We hypothesized strong deer effects on *C. ladanifer* size, associated with effects on seed production, and direct predation of flower-buds and developed fruits. Furthermore, we expected that in the long-term ungulate herbivory would limit *C. ladanifer* population compared 4) adult shrub densities in ungulate-exposed and ungulate-excluded plots after 12-years of experimental exclusion.

3.3 Methods

Study site

The study area is located in Tapada Real de Vila Viçosa (Tapada de Baixo) in southeast Portugal (38°48’N, 07°24’W). This is a 900 ha enclosed estate, dominantly covered by cork (*Quercus suber* L.) and holm (*Q. ilex* ssp. *rotundifolia* Lam.) oak, exploited for cork and primarily managed for deer hunting (e.g. food-supplementation, selective culling). The climate is typically Mediterranean, characterized by hot and dry summers and cool and wet winters. Mean annual precipitation is 585.3 mm mainly falling between October and May. The mean annual temperature is 15.9°C with a maximum of 31.1°C (in July) and a minimum of 5.8°C (in January) (Évora meteorological station, 1981–2010, http://www.ipma.pt, accessed in January 2013). The evergreen cork and holm oak woodland is relatively open (30 to 50 trees per ha), with an almost mono-specific understory of the shrub *C. ladanifer* L. interspersed with annual grasslands (Bugalho et al. 2011). The site is browsed by herbivore ungulates, red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) which, due to a limited culling policy and supplementary feeding in years of lower food availability, have been maintained in the study area at population densities of 0.35 and 0.1 deer per ha respectively. Although not uncommon in Iberian Peninsula hunting estates, such population densities are generally considered as high (Vargas et al. 1995, Acevedo et al. 2008). Few wild boars (*Sus scrofa*, L.) occurred at the beginning of the experiment, although they have locally thrived in recent years.

Cistus ladanifer, a woody perennial shrub, common to the western Mediterranean Basin, including southern Europe and northern Africa, is an obligate seeder (Guzmán and Vargas 2009). Fruits are globular lignified capsules which may contain between 500 to 1000 hard seeds. Flowering occurs between March and April followed by fruit maturation between May and July. Release of seeds starts in mid-summer and extends until the end of summer to the beginning of autumn (Talavera et al. 1993, Bastida and Talavera 2002). Evergreen or summer semi-deciduous shrubs, such as *C. ladanifer*, can be an important source of protein for herbivores during winter (January to April) and during the late Mediterranean summer to beginning of autumn (July to October), when most grasses are senescent and of low nutritive value (Bugalho and Milne 2003, Papanastasis et al. 2008). Thus, throughout the year, deer eats *C. ladanifer* young shoots and leaves but also buds, flowers and developed capsules (Bugalho and Milne 2003, Azorit et al. 2012) promoting dispersion and germination of *C. ladanifer* seeds (Malo and Suárez 1995).

Experimental design

In July 2001, 5 blocks of paired fenced (ungulate-excluded) and unfenced (ungulate-exposed) plots of 25 m × 25 m were randomly established in homogeneous areas of grassland, with no presence of *C. ladanifer* adults or juveniles. *C. ladanifer* shrubs had been previously cleared from these areas as part of common management practices in the estate. In Mediterranean cork and holm oak woodlands, shrubs are mechanically cleared each 4 to 7 years, rotationally in different locations, to prevent wildfires. Fences were 2.20 m tall for ensuring ungulate exclusion. Distance between paired (fence and unfenced) plots was approximately 25 m and between adjacent pairs between 250 m to 400 m. One open plot was lost in July 2004 and, thus, we considered data from the remaining four pairs of plots.

Long-term effects of ungulates on size of C. ladanifer

For estimating overall ungulate herbivory pressure (i.e. on both vegetative and reproductive plant structures), we compared the volume of *C. ladanifer* shrubs in ungulate-excluded and ungulate-exposed plots. To this end, we randomly selected 15 (in 2007) and 4 to 6 (in 2008 and 2013, respectively) *C. ladanifer* individuals in ungulate-exposed (open) and ungulate-excluded plots (fenced). Height and diameter of canopy projection of each sampled individual shrub was measured and shrub volume was estimated assuming the shape of an elliptical cone given by:

$$V = \left(\frac{1}{3}\right) \times \pi \times \frac{D1}{2} \times \frac{D2}{2} \times H$$

in which V is the volume of the shrub, D1 is maximum diameter of shrub canopy projection, D2 is diameter perpendicular to D1 and H is maximum shrub height.

To rule out the possibility that potential differences in plant individual size (shrub volume) and flower bud production between ungulate exclusion treatments were related to shrub age differences, we randomly selected and cut to ground level 4 shrubs in each plot in April 2008. Age of each individual shrub was then estimated through annual growth rings count. Growth rings were counted at the base of the trunk after buffing the surface with a high grade sand paper (Paton et al. 1998). We found no significant differences in age between *C. ladanifer* individuals in ungulate excluded (6.06 ± 0.11 years; mean ± s.e.m.) vs. ungulate exposed plots (5.71 ± 0.14 years; Mann Whitney U-test, U = 93, *P* = 0.127).

Effects of ungulates on buds and developed capsules

We monitored 60 and 60 (2007), 19 and 16 (2008), 40 and 12 (2013) *C. ladanifer* individuals in ungulate-exposed and ungulate-excluded plots, respectively. We recorded and compared in April of each year the number of buds (plus open flowers) remaining in ungulate-exposed and ungulate-excluded plots. To estimate the potential of ungulates to act as seed dispersers, during July and October of 2007 (i.e. when most capsules were ripe), we estimated the percentage of developed capsules removed by ungulates relative to the number of available buds or capsules in April and July, respectively. Although we did not directly confirm that ingestion of fully developed seeds leads to their dispersal, there is strong evidence that ungulates, and deer in particular, can act as seed dispersers when ingesting mature plant fruits (e.g. Malo and Suárez 1995). Because of the lack of teeth in front upper jaw, browsing marks left by deer (i.e. ragged edge on damaged stems) can be easily distinguished from browsing damage left by other herbivores such as rabbits, hares or voles (i.e. leave sharp-angled, knife-like cut on ends of stems) which sporadically occur in the area. Aborted fruits remained on *C. ladanifer* branches and were easily distinguished from normally developed fruits in ungulate-excluded and ungulate-exposed plots. Thus we only recorded differences between treatments in normally developed flower-buds and developed capsules at each sampling date.

Effects of ungulates on seed numbers, seed weight and germination

We randomly selected 5 shrubs per plot and collected 6 and 3 fruit capsules from each of these shrubs in July 2007 and July 2008, respectively. We collected *C. ladanifer* capsules without any signs of predation in July when seeds are mature. Capsules were then conserved at 4°C in a freezer. We estimated seed weight and number of seeds per capsule by weighing 100 seeds that were previously oven-dried at 60°C during 72 h. These seeds were randomly taken from 20 capsules collected in ungulate-exposed and 20 capsules collected in ungulate-excluded plots. Overall, 120 and 60 capsules per treatment were collected in 2007 and 2008, respectively.

For estimating seed germination we made composite seed samples per shrub using seeds from capsules collected in July 2008. Four replicates of 25 seeds each were then taken from each composite sample (2000 seeds per treatment overall) and seeds were placed in an oven at 100°C during 5 minutes to break dormancy (Delgado et al. 2001). Seeds were then distributed on filter paper disks (Whatman #1, n° 1001 125, GE Healthcare, Buckinghamshire, UK), randomly placed on modified Jacobsen individual apparatus trays (Willan 1991) in a germination incubator. The germination incubator was kept at a constant temperature (20°C) and under a 16 h-light photoperiod. Photosynthetic Photon Flux Density of 140 micromolm-2.s-1 was provided and measured with a Quantum Radiometer (Model LI-170, Li-Cor, Lincoln, N.E., USA). Every 3 days we counted and removed germinated seeds and seeds damaged by fungi from trays to avoid contamination of other seeds. Seeds were recorded as germinated as soon as the radicle emerged. The experiment was conducted over 105 days after which period no further germination was observed.

Effect of ungulates on soil seed bank

Soil seed bank was estimated by the seedling emergence method (Roberts 1981). Soil samples were collected at the end of October 2010 (after 9 years of ungulate exclusion) at the beginning of the germination period (Talavera et al. 1993). We randomly collected 18 soil cores per plot (0.05 m in diameter × 0.05 m in height) in each of the ungulate-excluded and ungulate-exposed plots. Samples were kept in the dark at 10°C, for 5 days, until the beginning of the emergence assay. We randomly placed homogenized soil samples in polyethylene containers (17 cm x 12 cm x 3 cm) and over a 1.5 cm bed of sterilized sand to allow seed germination. Soil was maintained moist, near field capacity, by an automatic irrigation system. We then identified, counted and removed emerged *C. ladanifer* seedlings from the containers, immediately after germination. Germinated seedlings of herbaceous species were removed to avoid competition with *C. ladanifer* seedlings. Germination trials lasted for 75 days until no more seed germination was observed.

Long-term ungulate induced changes in the population density of C. ladanifer

To estimate the long-term effect of ungulate activity on the population density of plants of *C. ladanifer*, we compared changes in shrub density in ungulate-exposed and ungulate-excluded plots after 12-years of experimental exclusion of ungulates. To this end, we randomly selected 18 (2 x 4 m) sub-plots within each plot (25 x 25 m) and recorded all individual adults of *C. ladanifer* during spring of 2007 and 2013. No *C. ladanifer* individuals occurred in any plot at the beginning of the experiment in 2001 (thus initial plot conditions could not affect our results). In 2007 all *C. ladanifer* individuals were alive both ungulate-exposed and ungulate-excluded plots. In 2013, however, dead individuals occurred in the plots and their numbers were also recorded.

Statistical analysis

Data on *C. ladanifer* bud and capsule numbers, shrub volume, seed weight and number, percentage of germination, number of emerged seedlings, and adult density were analyzed fitting generalized linear mixed models using Proc Glimmix in SAS (Littell et al. 2006). The effects of ungulate exclusion and year, as well as their interaction, were specified in the models as fixed effects, whereas the experimental plot and replicate (nested within plot) were included as random factors. A significant interaction between ungulate exclusion and year would indicate temporal inconsistency in the effect of herbivores on *C. ladanifer* performance. For proportions of seed germination, we specified in the corresponding models binomial error and logit-link function (see Littell et al. 2006). Because of high number of zero values in count response variables such as the number of emerged seedlings from soil samples and the number of adult *C. ladanifer*, we specified negative binomial (instead of Poisson) error and log-link function. For shrub volume we specified normal error and identity-link function. When the interaction between ungulate exclusion and year was significant, we performed tests for the effect of a given factor at the different levels of the other factor (“tests of simple main effects”), using the SLICE option in the LSMEANS statement of the MIXED procedure (Littell et al. 2006). For comparing number of dead individual *C. ladanifer* shrubs between treatments in 2013 we used Mann-Whitney tests (Zar 1996).

3.4 Results

Effects of ungulates on C. ladanifer size and number of flower buds

Cistus ladanifer volume in ungulate-excluded plots was on average 33.6 times larger as compared with shrubs within ungulate-exposed plots ($F_{1,198} = 197.09$, $P < 0.0001$; Fig 1A).

The differences were consistent among the three years, as indicated by the non-significant interaction between ungulate exclusion and year ($F_{2,198} = 0.61$, $P = 0.542$). We did not find significant differences in *C. ladanifer* volume among years ($F_{2,198} = 0.98$, $P = 0.378$; Fig 1A). Our generalized linear mixed model indicated that, once the effect of the random factor (i.e. plot) was controlled for, year had a significant effect as main factor on the number of *C. ladanifer* flower-buds ($F_{1,148} = 32.85$, $P < 0.0001$) being, on average, over 3-fold higher in 2008 than in 2007 (Fig 1B). As expected, ungulate exclusion also had a strong significant effect on number of flower buds ($F_{1,148} = 269.51$, $P < 0.0001$) being, on average, 27.3-fold higher in ungulate-excluded as compared to ungulate-exposed plots (Fig 1B). There was also a significant interaction between ungulate exclusion and year ($F_{1,148} = 22.31$, $P < 0.0001$), indicating that the effect of ungulate exclusion on *C. ladanifer* flower bud number was stronger during 2007 than during 2008 (Fig 1B). Given that during 2013 no individuals in ungulate-exposed plots (n = 40) produced buds, 2013 data was analyzed separately. Whereas all 12 individuals within ungulate-excluded plots produced abundant buds (Fig 1B), none individual in the ungulate-exposed plots produce any bud ($\chi^2 = 52.0$, df = 1, $P < 0.0001$). Thus, 2013 data confirmed the trend revealed in the previous two years, i.e. strong negative ungulate effects on number of *C. ladanifer* flower-buds.

Effects of ungulates on developed capsules

Our monitoring of bud fate during 2007 revealed that, as expected, the percentage of buds counted in April and remaining in July as developed capsules was 5.3-fold higher in ungulate-excluded plots as compared to ungulate-exposed plots ($F_{1,82} = 347.75$, $P < 0.0001$; Fig 1C). Similarly, the percentage of capsules counted in July that remained in October was 7.7-fold higher in ungulate-excluded plots as compared to ungulate-exposed plots ($F_{1,68} = 130.87$, $P < 0.0001$; Fig 1C). Overall, these results show sizable levels of ungulate consumption of developed capsules, indicating that they may act as potential seed dispersers.

Effects of ungulates on seed weight and number, soil seed bank and germination

Our mixed model indicated that, once the effect of plot was corrected for, seedling emergence in soil samples from ungulate-excluded plots was 2.6 times higher than in ungulate-exposed plots (F1,75 = 8.15, P < 0.01; Fig 2A).

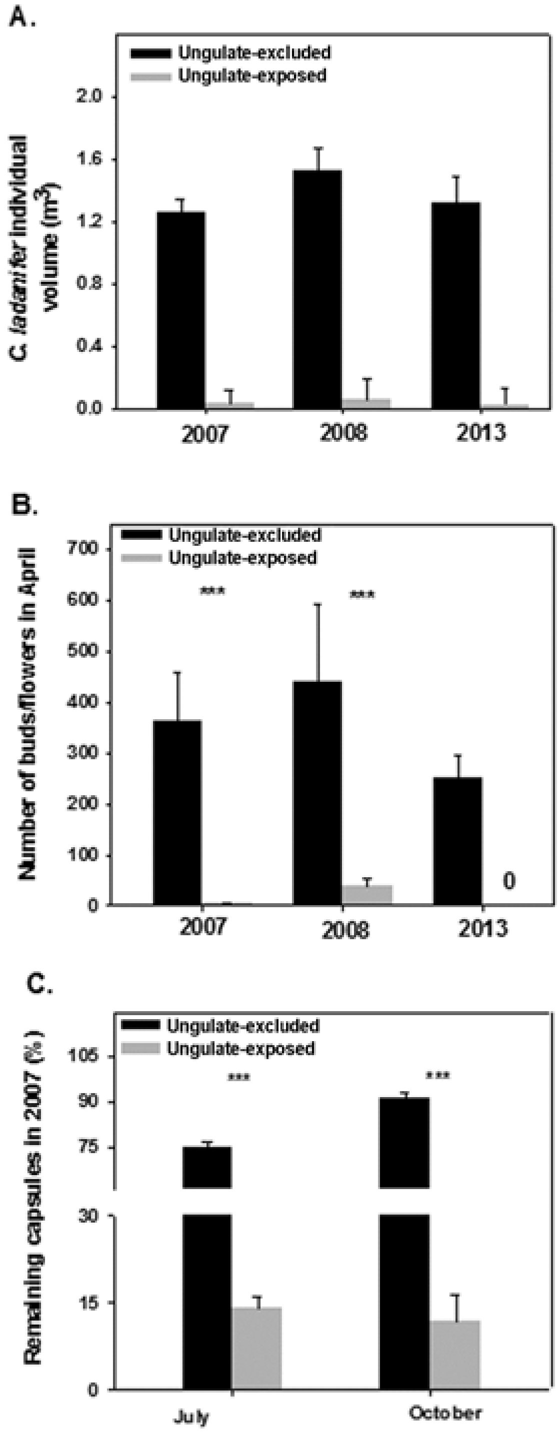

Fig 1. Model-adjusted means (± 1 SE) of A) *Cistus ladanifer* volume per individual in 2007, 2008 and 2013 B) Numbers of flower buds (plus open flowers) produced during 2007, 2008, and 2013 seasons in ungulate-excluded and ungulate-exposed (open) plots and C) Percentages of remaining capsules in July (regarding flower bud number in April) and October 2007 (regarding capsule number in July). (***, *P* < 0.001)

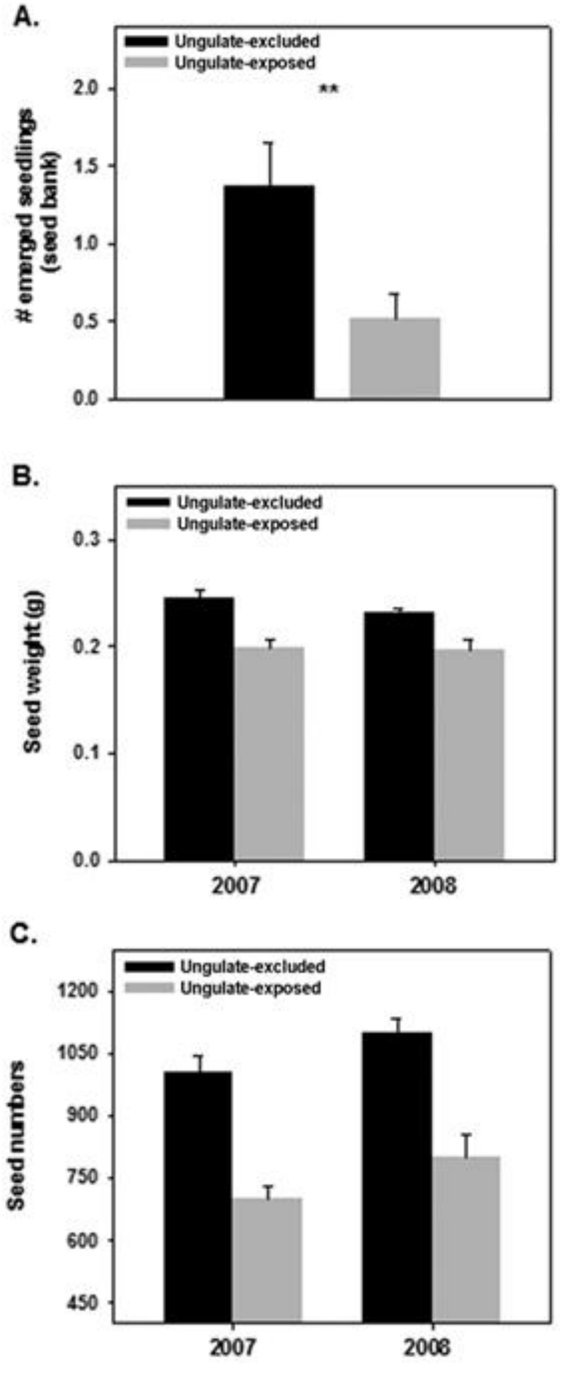

Fig 2. Model-adjusted means (± 1 SE) of A) Number of *Cistus. ladanifer* seedlings emerged from soil seed bank samples B) Seed weight and C) Seed numbers from capsules collected in ungulate-excluded and ungulate-exposed (open) plots (**, P < 0.01; ***, P < 0.001; and ns, not significant [P > 0.05]).

Our chamber experiment revealed that, seed germination did not vary between ungulate-excluded and ungulate-exposed plots ($F_{1,143} = 0.45$, $P = 0.502$; Fig 2B) in spite of seed weight being significantly higher in both years in ungulate excluded plots (seed weight in ungulate-excluded and ungulate-exposed plots, respectively: 0.245 ± 0.007 vs. 0.198 ± 0.008 mg in 2007 and 0.231 ±0.004 vs. 0.197 ± 0.009 mg in 2008, $F_{1,119} = 11.19$, $P < 0.001$) (Fig 2C). Overall, these results showed a higher seedling emergence in ungulate-excluded soil samples, in spite of no differences in seed germination between treatments. These results are also consistent with a higher number of seeds per capsule found in ungulate-excluded plots (seed numbers per capsule in ungulate-excluded and ungulate-exposed plots, respectively: 1006.20 ± 36.63 vs. 701.88 ± 29.85 seeds per plot in 2007 and 1100.07 ± 33.32 vs. 798.45 ± 57.61 seeds per plot in 2008, F1,307 = 30.41, P < 0.001) (Fig 2C).

Effects of ungulates on the population density of C. ladanifer

Although no *C. ladanifer* individual occurred in our experimental plots in 2001, colonization started soon after the field experiment was initiated (Authors personal observation). Ungulate exclusion had a strong significant effect as main factor on *C. ladanifer* density ($F_{1,281} = 51.86$, $P < 0.0001$) which was, on average, over 3.8-fold higher in ungulate-excluded as compared with ungulate-exposed plots (Fig 3). Year (2007 and 2013) did not have an effect as main factor ($P = 0.109$), but it showed a significant interaction with ungulate exclusion ($F_{1,281} = 22.89$, $P < 0.0001$) indicating that the sign and/or strength of its effect on the population density of *C. ladanifer* varied between ungulate-exposed and ungulate-excluded plots (Fig 3). Specifically, tests of slices revealed that whereas *C. ladanifer* population density in ungulate-excluded plots increased during the five experimental years ($F_{1,281} = 5.69$, $P < 0.05$), it clearly shrank in ungulate-exposed plots during such time period ($F_{1,148} = 18.37$, $P < 0.0001$; Fig 3). Furthermore, whereas no *C. ladanifer* individuals occurred in any of the plots in 2001, and whereas *C. ladanifer* population density did not significantly differ between ungulate-exposed and ungulate-excluded in 2007 ($F_{1,281} = 3.14$, $P = 0.077$), at the end of the experiment *C. ladanifer* population density was significantly lower in ungulate-exposed plots ($F_{1,281} = 67.40$, $P = 0.0001$; Fig 3). Additionally a higher number of dead individuals was recorded in ungulate exposed plots in 2013 (4.57 ± 0.55 vs. 7.38 ± 0.79 dead individuals/plot, in ungulate-exposed vs. ungulate-excluded plots, respectively, *P* < 0.0001, Mann-Whitney U test, U = 58.5).

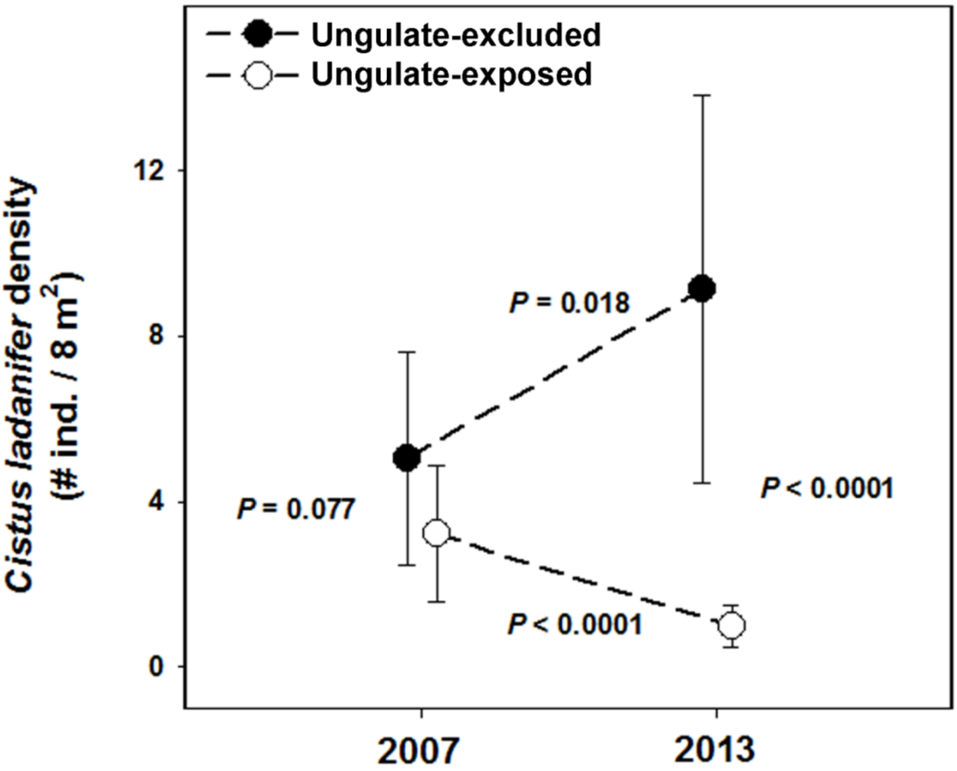

Fig 3. Changes in *C. ladanifer* population density (shrubs per 8m2) in 2007 and 2013 in the ungulate-excluded and ungulate-exposed plots. Because the interaction between ungulate exclusion and year was significant, we report the *P*-values of the tests for the four simple main effects involved in the interaction

3.5 Discussion

Plant-animal interactions imply both costs and benefits for the participants (Fedriani et al. 2012, Chamberlain et al. 2014) that may ultimately affect their population densities (Anderson and Holte 1981, Augustine and McNaughton 2004). Our study showed that high density populations of ungulate herbivores can have a drastic negative effect on plant size and number of *C. ladanifer* flower-buds produced thus, limiting the availability of ripe fruits holding fully developed seeds that could be disseminated into the soil seed bank. The effects of such dramatic decline of shrub reproductive structures, translated along the plant reproductive cycle and led to reduced fruit set, limited soil seed bank, ultimately affecting the population density of *C. ladanifer*. The long-term net balance between ungulate herbivory and its effects on number of flower buds and potential seed dispersal was thus highly negative for the plant, as illustrated by the strong decrease of *C. ladanifer* density after 12 years. These results highlight the potential of high density ungulate populations, favored by habitat management practices such as limited culling policies and food supplementing, to alter the nature of species interactions (Kiers et al. 2010, Fedriani et al. 2013, Traveset and Richardson 2014).

Individual and long-term population effects of C. ladanifer - ungulate interactions

Although strong negative effects of ungulates on plant reproductive success (Allison 1990, Gómez 2005, Goheen et al. 2007, Haarmeyer et al. 2010) or on growth and survival (Mathisen et al. 2010, White 2012, Sankaran et al. 2013, Pringle et al. 2014) have been often documented, few studies have assessed the population consequences of these interactions (but see Pringle et al. 2014). Our long-term ungulate-exclusion revealed that cumulative effects of ungulates ultimately led to a decline of *C. ladanifer* population density, possibly by decreasing the seed bank. Although depletion of seed banks, as affected by heavy grazing, has been shown in grasslands (O'Connor and Pickett 1992, DiTommaso et al. 2014), less is known for woody plant communities, for which browsing and grazing have been mainly shown to alter plant succession (Beauchamp et al. 2013, DiTommaso et al. 2014). In our study, we have shown that ungulate herbivory on *C. ladanifer* led to a noticeable soil seed bank depression after 9 years. Given that persistent soil seed banks are especially critical in highly climatic variable ecosystems (Baskin and Baskin 2014), such as Mediterranean ecosystems (Thompson 2005), this is likely to negatively affect *C. ladanifer* dynamics and resilience, which seeds can persist viable in the soil seed bank for 6 to 7 years (Clemente et al. 1996, 2007). Furthermore, although herbivores can confer plants with benefits such as over-growth (Oba et al. 2000, Haarmeyer et al. 2010) or seed dissemination (Malo and Suárez 1995, Pringle et al. 2014), very few studies have assessed separately negative and positive effects of herbivores on plants. Here, we have shown strong negative effects of ungulate herbivores on *C. ladanifer* plant size and reproductive structures, but also considerable ungulate ingestion of ripe capsules comprising fully developed seeds, which were likely to being dispersed (Malo and Suárez 1995). Our results, suggest that ungulate herbivory seems to have overridden potential positive effects of ungulates on *C. ladanifer* seed dissemination. Moreover, our conclusions are probably conservative as our experimental setting prevented any arrival of dispersed seeds in the ungulate-excluded plots but not in the ungulate-exposed plots.

The strong net negative cumulative effect of ungulates on the population density of *C. ladanifer* may have been exacerbated by synergistic effects of ungulate herbivory and drought (Webster et al. 2008). Indeed, 2012 was an extremely dry year (Caldeira et al. 2015) and the higher mortality of *C. ladanifer* observed in ungulate-exposed plots in subsequent year may have resulted from herbivory and drought interactions. Interestingly, our results also suggest a considerable lag effect of herbivory on *C. ladanifer* population density. These results highlight the importance of long-term field experiments when investigating the population outcomes of plant-animal interactions (e.g. Gómez 2005). Results also emphasize that only long term studies, such as ours, can properly capture the effects of high inter-annual climatic variability, such as that typical of Mediterranean environments, when investigating species interactions.

Context-dependency of plant-ungulate interactions and community-level effects

Variation in the strength and sign of plant-herbivore interactions are likely to occur as a result of changes in the ecological context (e.g. Holland & DeAngelis 2009, Fedriani et al. 2012, Chamberlain et al. 2014). The intensive ungulate consumption of *C. ladanifer*, a species often considered as unpalatable (Miranda et al. 2011), during this and previous studies (Bugalho et al. 2001, Bugalho and Milne 2003) is likely to be related to the generalist feeding habits of target consumers, which tend to feed on woody and grass species according to plant food availability (Hofmann 1989). The high ungulate population densities during the period of our study probably enhanced food competition and forced animals to become less selective, leading to a higher consumption of *C. ladanifer* (Miranda et al. 2011). Also, when availability and quality of grasses is low, browsing pressure on *C. ladanifer* is likely to increase and to occur early in the season, as compared with years of higher availability of grasses (Seligman 1996, Bugalho and Milne 2003). This will exacerbate negative effects on shrub plant size and number of flower buds produced. Further long-term research to unravel the extent to which variation in the strength and sign of *C. ladanifer*-ungulate interactions relate to bud production or to third party factors such as availability of alternative plant food, such as grasses, is certainly needed.

The intensive interaction between high-density ungulate populations and *C. ladanifer* can, in addition, cascade through the whole ecosystem (Schmitz et al. 2000, Palmer et al. 2008) affecting biodiversity and ecosystem services delivery. For example, because ungulates can dramatically reduce *C. ladanifer* population densities, which is a shrub species that strongly competes with herbs and grasses in evergreen oak woodlands, in areas where ungulates are limiting the expansion of *C. ladanifer* populations, they may be indirectly favoring grass abundance and diversity (Diaz-Vila et al. 2003). Also, by decreasing shrub population densities, as well as individual shrub volume and thus biomass (Fig 1A), high density ungulate populations are likely to lessen the risk and severity of wildfires, an important ecosystem service in several human-shaped Mediterranean ecosystems (Bugalho et al. 2011).

Our long-term experimental study illustrates how high population densities of ungulates, favored either by habitat management (e.g. food-supplementation, under-harvesting) or land use changes that increase the availability of suitable habitat (Gordon et al. 2004), may alter the balance between costs and benefits in plant-animal interactions. Further research on the community and whole ecosystem-level effects of over-abundant ungulate populations are certainly needed to forecast the outcomes of global change in species interactions and ecosystem ecology.

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CHAPTER 4

LONG-TERM EFFECTS OF DEER BROWSING ON THE TRADE-OFF BETWEEN ABOVE-GROUND CARBON STOCK AND FIRE HAZARD

MANUSCRIPT: Lecomte X., Caldeira M.C., Catry F.X., Fernandes P.M., Jackson R.B., Bugalho M.N.. 2017. Ecosystem trade-offs in a Mediterranean oak ecosystem: May ungulate browsing increase long-term C stocks through reduction of fire hazard?

4.1 Abstract

Ungulates influence ecosystem services in important ways, including altering the amount of standing biomass and species composition. Browsing, for example, removes biomass and, in consequence, can decrease the risk of wildfires in some systems. The influence over ungulates on carbon (C) storage is more complicated. Browsers reduce C stocks directly by consuming biomass, but if browsing reduces fine fuel loads, then long-term C storage may actually increase. We investigated how wild ungulates mediate trade-offs between C stocks and wildfire hazard in a Mediterranean oak woodland. We assessed the effects of deer (*Cervus elaphus* and *Dama dama*) on the encroachment of gum cistus (*Cistus ladanifer*), a highly flammable Mediterranean shrub, through a long-term ungulate exclusion experiment. We compared C stocks and fire behavior between *C. ladanifer* stands either continuously browsed or excluded from browsing for 14 years. We estimated plant biomass and experimental fire behavior in control and exclusion plots. Ungulates reduced above-ground C stocks of the shrub and litter components by nearly 90%, but did not affect C stocks of oak trees. Ungulates also significantly reduced shrub density, biomass and height by 89% ($P < 0.01$), 97% ($P < 0.001$) and 87% ($P < 0.001$), respectively ($P = 0.01$, $P < 0.001$ and $P < 0.001$ respectively), and concomitantly altered fire behavior. Browsing reduced vegetation total fine fuel load, an important contributor to fire spread, by 80% ($P < 0.001$). In browsed plots, wildfire rate of spread, flame length, and fireline intensity decreased by 50%, 65%, and 90%, respectively, which decreased likelihood of adult oak mortality. By decreasing fire hazard, ungulates could ultimately contribute to increase ecosystem C stocks through increased tree survival. These are indirect critical trade-offs that need to be assessed when addressing management and effects of ungulates on the ecology of ecosystems.

Keywords: above-ground biomass, shrub encroachment, fuel management, fire hazard, ungulates, browsing

4.2 Introduction

Woody encroachment into grasslands has been increasingly documented worldwide (Sala and Maestre 2014) and associated with climate and land-use changes, including altered fire and grazing regimes (Briggs et al. 2005, Sankaran et al. 2008, D'Odorico et al. 2012). Shrublands provide a wide range of ecosystem services, including C storage and sequestration and livestock forage (Pacala et al. 2001, Janssens et al. 2003, Piao et al. 2009, McKinley et al. 2011). Increased C stocks however, may trade-off with increased wildfire hazard, particularly in Mediterranean environments (Fernandes 2009). Although dependent on factors such as precipitation (Jackson et al. 2002), increased net primary productivity, biomass accumulation, and consequent increase of C stocks can be significant in shrub encroached ecosystems and an important shrubland ecosystem service (Hughes et al. 2006). For example, aboveground C stocks in six shrublands along a climate gradient in Europe ranged from 1.4 to 18.2 Mg C ha-1 (Beier et al. 2009) and in a dryland ecosystem, in northern Texas, USA, C stocks increased from 3.8 to 5 Mg C ha-1, during 60 years of *Prosopis glandulosa* encroachment (Asner et al. 2003, Archer and Predick 2014). Trees, however, are the major C stock component of the ecosystems (Pan et al. 2011, Zomer et al. 2016). Mediterranean Basin oak woodlands, for example, with a tree cover of 30%, may sequester up to 140 g C m–2 yr–1 (Pereira et al. 2007), a value within the range of savannas in California (Baldocchi and Xu 2004), which contribute to high ecosystem C stocks. The decrease of plant moisture content during the warm and dry summer (Dennison et al. 2003, Pellizzaro et al. 2007, Dennison and Moritz 2009) however, increases shrub flammability, contributing to a high fire hazard of Mediterranean shrublands (Pausas and Bradstock 2007, Keeley 2013, Rego and Silva 2014). Additionally, fire behavior is strongly dependent on the properties and structure of vegetation, e.g. rate of fire spread and fire intensity increase with shrub height and density (Fernandes 2001, De Luis et al. 2004, Anderson et al. 2015(Fernandes 2001, De Luis et al. 2004).

Woody plants are important sources of forage for large ruminant herbivores, especially in drylands, such as in Mediterranean environments (Bugalho and Milne 2003, Papanastasis et al. 2008, Azorit et al. 2012). Therefore, fuel loads can be effectively reduced in fire-prone landscapes through grazing and browsing (Briggs et al. 2005, Hobbs 2006). By ingestion and trampling, livestock affects the horizontal and vertical structure of shrublands and reduces the amount of fine fuel loads (Nader et al. 2007, Ingram et al. 2013, Strand et al. 2014, Davies et al. 2016). This reduces the probability of ignition, and the spread and intensity of wildfires (Campbell and Taylor, 2006). Wild and domestic ungulates, such as deer or goats, were shown to disrupt fuel ladders, breaking the vertical continuity of biomass between ground and tree canopy, and decreasing litter depth and fine fuel loads in Mediterranean areas (Lovreglio et al. 2014) and therefore decreasing wildfire hazard.

Here we investigated how wild ungulates mediate trade-offs between the ecosystem services of fire hazard mitigation and increased C stocks in a Mediterranean oak ecosystem. As part of a 14-year exclusion experiment, we measured the long-term effects of red (*Cervus elaphus*) and fallow deer (*Dama dama*) on the encroachment of gum cistus (*Cistus ladanifer*), a fire-prone Mediterranean shrub, also investigating how deer affected C stocks and wildfire hazard. More specifically, we compared C stocks and wildfire behavior between oak stands encroached by *C. ladanifer* that were browsed or protected from ungulates over the last 14 years. We hypothesized that protection from deer increased 1) average plant height, volume and density of *C. ladanifer* stands; 2) total above-ground biomass and C stocks, namely of *C. ladanifer* and 3) fine fuel load accumulation. Finally, we hypothesized that ungulates decreased potential fire spread, intensity, severity, and the probability of crown fires and oak mortality and ultimately indirectly benefited long-term ecosystem C stocks (Fig. 1).

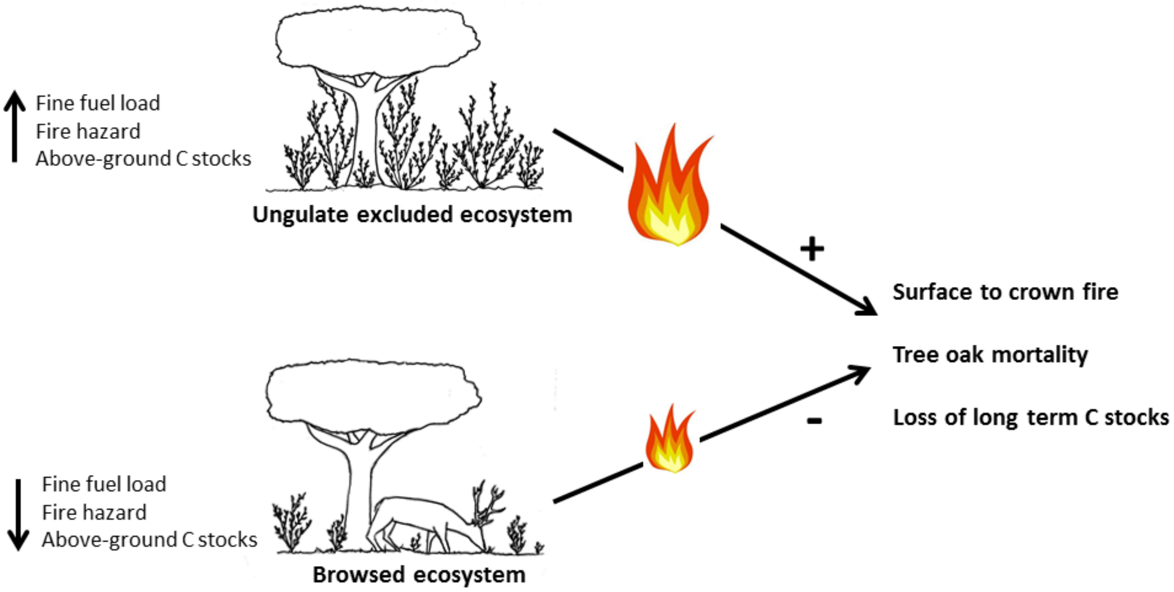

Figure 1. Conceptual model depicting analyzed oak forest community interactions and long-term ecosystem services trade-offs provided by ungulate herbivory. Ungulate browsing is expected to alter understory vegetation (i.e. spatial structure and biomass accumulation), reducing long-term carbon storage and fine fuel load (i.e. shrub, litter and herbs). This may mitigate fire hazard and behavior (Fernandes 2009), especially surface to crown fire transition, reducing tree mortality. Ungulate browsing is expected to reduce loss of long-term carbon storage. The relative strength of these interactions may vary in space and time in accordance with ungulate density and feeding resources (e.g. grass, acorns) availability

4.3 Methods

Site and species

The experiment was conducted in Tapada Real de Vila Viçosa (Tapada de Baixo), an enclosed estate of 900 ha, located in southeast Portugal (38°49’N, 07°24’W). The study site was an open woodland of evergreen holm (*Quercus rotundifolia*) and cork oak (*Q. suber*). Woodland understory was dominated by *C. ladanifer*, a semi-deciduous Mediterranean shrub species, and annual grasslands were primarily composed of grasses (e.g. *Bromus madritensis*, *Gaudinia fragilis*), with some forbs (e.g. *Andryala integrifolia*, *Leontodon taraxacoides*) and legumes (e.g. *Vicia disperma*, *Ornithopus compressus*) occurring in smaller proportions. *C. ladanifer* is a highly flammable species because of the terpene-derived resin that impregnates its leaves and branches (Núnez-Regueira et al. 2005), which may also lessen litter decomposition and accelerate fine fuel accumulation (Horner et al. 1988). Population densities of red and fallow deer, estimated at the beginning of the experiment, were 0.4 and 0.1 individuals per ha, respectively. The climate is typically Mediterranean with hot and dry summers and cool and wet winters. Mean annual precipitation is 585 mm mostly distributed between October and May and mean annual temperature is 15.9 ºC (maximum of 31.1 ºC in July and minimum of 5.8ºC in January).

Experimental design and sampling

We established 5 blocks of paired ungulate-excluded and ungulate-exposed (control) plots of 25 m × 25 m in July 2001. Ungulate-excluded plots were fenced with a 2.20 m tall fence to exclude browsing by deer. Plots were established in homogeneous areas of grassland, where *C. ladanifer* was absent due to mechanically clearing, through ploughing, as part of the management practices of the estate. There were between 2 and 3 cork and 2 and 3 holm oaks in each open and fenced plot. Each 25 m x 25 m plot was further divided into 40 sub-plots of 2 m x 4 m and 18 of these sub-plots were randomly selected for *C. ladanifer* measurements. In July 2004 one open plot was lost because of unintentionally ploughing, hence only the remaining 4 pairs of plots were used in data analysis. After 2003 *C. ladanifer* started colonizing browsed and un-browsed plots.

Shrub layer structure and biomass

In March 2007 and 2015, we measured maximum height of 40 randomly selected *C. ladanifer* individuals in ungulate-excluded and ungulate-exposed plots and counted all *C. ladanifer* dead and living individuals occurring in the 18 randomized sub-plots. Furthermore, in 2015 we also measured the basal trunk diameter of *C. ladanifer* individuals occurring in the sub-plots.

To estimate above-ground biomass of *C. ladanifer* stands, we used plant biomass-volume allometric equations as follows. In 2007, we randomly tagged 15 *C. ladanifer* individuals per plot (60 individuals per browsing treatment). We then estimated *C. ladanifer* volume for all tagged individuals, assuming an elliptical cone shape for the species:

$$V = (1/3) \times \pi \times (D1/2) \times (D2/2) \times H$$

where V was the volume of the shrub (m^3), D1 the maximum diameter of shrub canopy projection (m), D2 the diameter perpendicular to D1 (m) and H the maximum shrub height (m). We estimated dry above-ground shrub biomass by cutting to ground level 16 and 19 of the total tagged shrubs in the ungulate-excluded plots and in the ungulate-exposed plots, respectively. We oven-dried samples at 80ºC to constant mass and weighed them. Biomass and volume were then related through allometric equations (e.g., Lecomte et al. 2016).

Because of a high number of juvenile plants of *C. ladanifer* observed in the ungulate-excluded plots in 2015, we estimated *C. ladanifer* above-ground biomass in a stratified manner. We randomly selected 6 to 10 *C. ladanifer* living individuals per plot (30 shrubs per treatment) according to 10 mm stem diameter classes (between 6 to 86 mm). We then estimated total aboveground biomass of each measured individual shrub using allometric equations and considering class stem diameters. We estimated biomass of dead shrubs in a similar way, excluding the biomass of leaves. The branch structure and volume of dead *C. ladanifer* individuals was similar to that of living individuals without leaves.

To assess *C. ladanifer* fuel load we separated shrub biomass into dead and living components by stem diameter classes for modeling fire behavior (Anderson 1982, 2009). These diameter classes, used to determine the time woody fuel takes to ignite, were: Live woody fuel, i.e. live components of shrubs including the foliage and very fine stems <0.64 cm in diameter; 1-h fuel, i.e. dead components including leaves, senesced herbaceous plants and fine dead stems <0.64 cm in diameter; 10-h fuel, i.e. dead components with 0.64-2.54 cm in diameter; and 100-h fuel, i.e. dead components with diameters of 2.54 to 7.62 cm.

Tree layer structure and biomass

We measured diameter at breast height, maximum height and height of the canopy base of all individual oak trees in the plots. In the case of cork oak, we also measured bark thickness of all cork oak trees using a bark gauge.

We estimated canopy area by measuring the longest (D1) and its perpendicular (D2) length projections on the ground considering an elliptical canopy shape (canopy area = $\pi \times (D1/2) \times (D2/2)$). We estimated canopy cover as the percentage of the plot occupied by the tree canopy projection using ImageJ software (version 1.51p, NIH, Maryland, USA). We calculated total above-ground oak biomass including biomass of leaves and of thin branches (diameter <2 cm) of cork and holm oak following the equations of Ruiz-Peinado et al. (2012). Canopy bulk density was used to determine fine fuels, i.e. biomass of canopy fuel with diameter <0.6 cm per unit of canopy volume (Scott and Reinhardt 2001). We estimated canopy bulk density of the plots by sampling a thin branch from 1 cork and 1 holm oak per plot and treatment (8 individuals per species). These branches were cut and sorted into diameter size class (i.e. < 0.6 cm and > 0.6 cm), oven-dried at 60 ºC to constant mass and weighed. Allometric equations between diameter at breast height (DBH) and total biomass of branches with a diameter <0.6 cm were then used to estimate canopy bulk density for cork and holm oaks (Appendix 1).

We measured biometric parameters of 49 oak trees (28 cork and 21 holm oaks) in all plots (population oak density of 98 ± 6 trees ha-1). We found no significant differences in biometric parameters, neither of oak trees in ungulate-exposed and ungulate-excluded plots, nor between cork and holm oak trees (Appendix 2 and 3). For the overall oak population mean canopy cover, mean canopy height, mean canopy base height, mean canopy bulk density and diameter at breast height were, respectively (mean ± sem): 59.9 ± 3.3 %, 8.0 ± 0. 2 m, 2.30 ± 0.09 m, 0.11 ± 0.02 kg m-3 and 42.4 ± 1.4 cm. Mean bark thickness of cork oak trees was 3.50 ± 0.02 cm.

Herb and litter biomass and overall C stocks

We estimated herb biomass during the first week of June 2007 and 2015, the peak of plant biomass production in our study area. We randomly selected 4 sub-plots and clipped to ground level all above-ground plant material within a 50 cm x 25 cm quadrat. For estimating litter biomass we collected all dead plant material within the 50 cm x 25 cm quadrat that was located in 8 randomly selected sub-plots. We separated the samples by diameter class, as described for shrub biomass and oven-dried (at 70^{o}C to constant mass) and weighed them. We estimated above-ground C stocks of *C. ladanifer* using the conversion factor of 0.51 g of C per g of dry biomass determined for *C. ladanifer* (Ruiz-Peinado et al. 2013), 0.50 g of C per dry biomass for herbs and litter (Fonseca et al. 2012) and 0.47 g of C per dry biomass for oaks (Gil et al. 2011).

Fire behavior and effects on trees

We simulated fire behavior in each browsing treatment using BehavePlus 5.0.5 fire modeling software (Andrews 2009). BehavePlus allows estimating fire behavior from fuel characteristics, weather, and slope as input data (Andrews 2014). We used shrub and tree cover and heights and total dead fuel load, from shrubs, herbs and litter, separated by time lag classes (i.e. 1-h, 10-h and 100-h, see above) and live woody fuel load as data input. We set temperature at 31 ºC, as a baseline, as wildfires usually occur in summer in Mediterranean regions. Herbs were included in the 1-h class as most grassland species in the study area are annuals that senesce and die during summer. Fuel moisture contents values were set at 4%, 5% and 6%, respectively for 1-h, 10-h, and 100-h dead fuels, and 75% and 80% for live woody fuels, respectively in the understory and in the overstory (Chuvieco et al. 2009, Fernandes 2009). We corrected total fuel bed height using an empirical model developed for shrublands (Burgan and Rothermel 1984) and used the BehavePlus modules SURFACE, CROWN and SCORCH to predict the fire behavior and the potential effects on oak trees. We performed simulations for a 0-30 km h-1 range of wind speeds aligned with a 5º slope, which is within the range of slopes for our plots (mean ± sem: 4.6 ± 0.5 º, range: 1.4º to 7.5º). Among the fire behavior outputs we reported flame length (m), surface rate of spread (m min^{-1}) and fireline intensity (MW m^{-1}).

We predicted oak tree mortality using the results of fire behavior simulations, along with tree characteristics and existing fire-effects models for cork and holm oaks (Catry et al. 2010, 2012, 2013). These models are based on tree biometrics (DBH, height, bark thickness) and fire severity descriptors (maximum char height expressed as percentage of total tree height). To estimate fire severity, we considered char height as flame length plus the height of the fuel bed. Additionally, when flame length was over 50% of tree height indicating a transition to crown fire and crown torching, we assumed that char height was 100%.

Statistical analysis

We used generalized linear mixed models to compare *C. ladanifer* population density, height and C stocks as well as herb and litter biomass, between ungulate-excluded and ungulate-exposed plots. Due to high number of zero values in *C. ladanifer* count for shrub density, we used a negative binomial error and log-link function (see Lecomte et al. 2016). For shrub height and aboveground C stocks, herbaceous C stocks, litter C stocks and shrub fuel load, we specified a normal error and identity-link function. Ungulate exclusion was specified in the models as fixed effect, as well as year and interaction between ungulate exclusion and year. Plot and replicate (nested within plot) were included as random factors. We fitted nonlinear regression models to determine the best relationship between volume and biomass (i.e. dry weight) of *C. ladanifer*. We used t-tests to compare tree canopy cover, tree canopy height, tree canopy bulk density, tree diameter at breast height, herbs and litter 1-h dead fuel load between ungulate-excluded and ungulate-exposed plots. We used a Mann-Whitney-U test to compare litter 10-h dead fuel load because of the lack of normality of distribution and homogeneity of variance. We conducted statistical analyses using SPSS® software package (PASW Statistics, v. 23.0.0.0, 2015).

4.4 Results

Effects of ungulates on the structure of C. ladanifer understory

Ungulates substantially altered the structure of *C. ladanifer* understory. Living shrub density, was on average greater in ungulate-excluded plots as compared to ungulate-exposed plots ($F_{1,284} = 7.371$, $P = 0.007$) (Fig. 2A). Year did not affect significantly shrub density ($F_{1,284} = 1.359$, $P = 0.245$) as a main factor, but there were marginally significant interactions between browsing treatment and year ($F_{1,284} = 2.768$, $P = 0.097$). Whereas ungulates did not affect population density of living *C. ladanifer* in 2007 ($F_{2,142} = 1.736$, $P = 0.180$), significant negative effects were found in 2015 ($F_{2,142} = 4.765$, $P = 0.010$), with higher shrub densities in the ungulate-excluded plots (Fig 2A). Surprisingly, while living *C. ladanifer* density increased in the ungulate-excluded plots between 2007 and 2015 ($F_{2,142} = 27.494$, $P < 0.001$), it decreased in the ungulate-exposed plots over the same period ($F_{2,143} = 3.463$, $P = 0.034$) (Fig 2A), highlighting the importance of ungulate ecology.

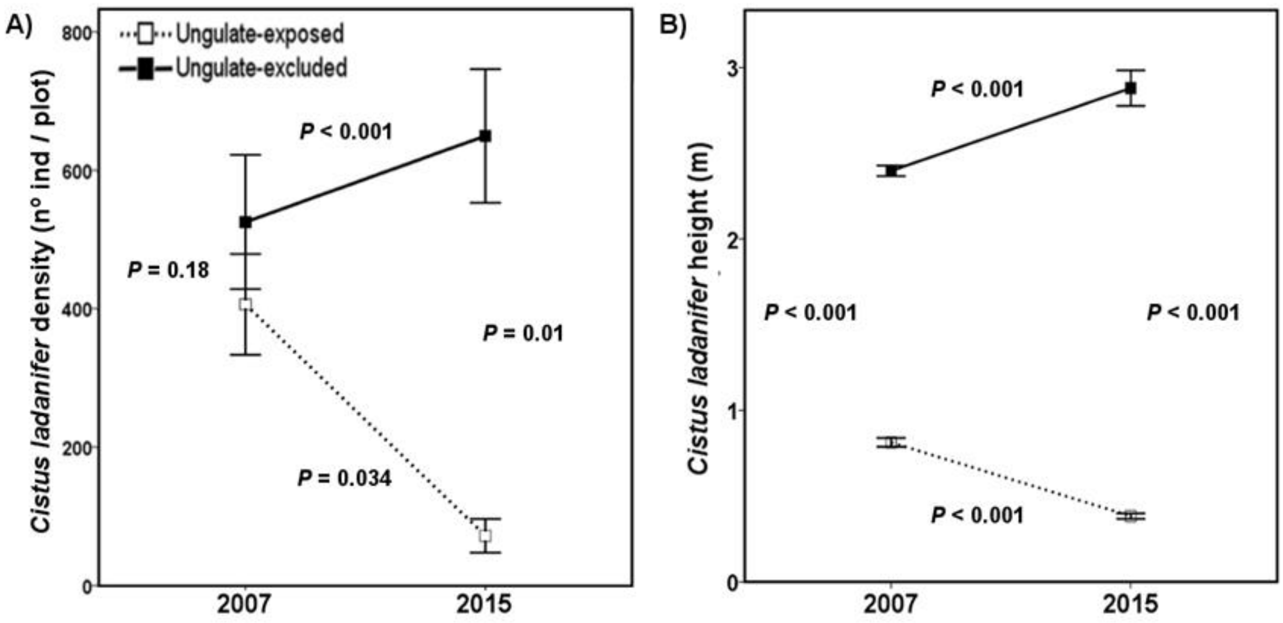

Figure 2. A) Density and B) height of living *C. ladanifer* in ungulate-excluded and ungulate-exposed plots in 2007 and 2015. (Mean ± sem). *P*-values are reported for the four single effects.

Shrub height was on average greater in ungulate-excluded compared to ungulate-exposed plots ($F_{1,156} = 153.38$, $P < 0.001$) (Fig. 2B). Year as a main factor did not affect shrub height ($F_{1,156} = 0.13$, $P = 0.723$), but there was a significant interaction between ungulate-exclusion and year ($F_{1,156} = 87.85$, $P < 0.001$). Indeed, height increased significantly in ungulate-excluded plots between 2007 and 2015 ($F_{2,78} = 216.21$, $P < 0.001$) but decreased in the ungulate-exposed plots ($F_{2,78} = 127.03$, $P < 0.001$) over the same period.

Effects of ungulates on above-ground C stocks

Ungulate exclusion had a strong effect on aboveground C stocks of *C. ladanifer* which were greater in the ungulate-excluded plots than in the ungulate-exposed plots ($F_{1,260}$ = 65.80, *P* < 0.001) (Fig. 3A). Year also affected *C. ladanifer* C stocks ($F_{1,260}$ = 4.96, *P* = 0.027), being on average, 2-fold greater in 2015 than in 2007. There was a significant interaction between ungulate exclusion and year ($F_{1.260}$ = 5.52, *P* = 0.020) with *C. ladanifer* C stocks increasing by 65% in the ungulate-excluded plots between 2007 and 2015 ($F_{2,130}$ = 48.41, *P* < 0.001) and decreasing by 40% in the ungulate-exposed plots during the same period ($F_{2.130}$ = 5.61, *P* = 0.05).

Ungulate exclusion did not affect herb above-ground C stocks (*P* = 0.264) (Fig. 3B), which varied significantly between years ($F_{1,60}$ = 142.92, *P* < 0.001), probably due to different climatic conditions in 2007 and 2015.

C stocks of litter were on average 2-fold greater in ungulate-excluded plots ($F_{1,124}$ = 8.79, *P* = 0.004) (Fig. 3C). Year also affected significantly litter C stocks ($F_{1,124}$ = 11.62, *P* = 0.001). From 2007 to 2015, litter C stocks increased by 25% and 35% in the ungulate-exposed and ungulate-excluded plots, respectively. There were no significant interactions between ungulate-exclusion and year (*P* = 0.144), indicating temporal consistency in the effect of ungulate exclusion on litter accumulation.

Ungulates affected strongly total understory aboveground C stocks, which were on average 6-fold greater in the ungulate-excluded plots ($F_{1.12}$ = 16.46, *P* = 0.02) (Fig. 4). Year also affected significantly total C stocks, showing increased ungulate exclusion effect between 2007 and 2015 ($F_{1,12}$ = 6.01, *P* = 0.03). Total C stocks increased in the ungulate-excluded plots by nearly 50% between 2007 and 2015 ($F_{2,6}$ = 21.47, *P* = 0.002) but decreased by 20% in the ungulate-exposed plots during the same period ($F_{2,6}$ = 55.42, *P* < 0.001), as shown by a significant interaction between ungulate exclusion and year ($F_{1,12}$ = 8.32, *P* = 0.014).

Ungulates did not affect tree oak above-ground C stocks (mean ± sem = 31.8 ± 4.1 Mg C / ha, t-test, t(6) = 1.33, P = 0.232) which corresponded to 67% and 94% of total above-ground C stocks in ungulate-excluded and ungulate-exposed plots, respectively. Overall, 14 years of browsing exclusion led to a 40% increase of the total aboveground C stocks (tree and understory C stocks; mean ± sem = 47.3 ± 2.2 Mg C / ha and 33.7 ± 0.2 Mg C / ha in ungulate-excluded and ungulate-exposed plots, respectively).

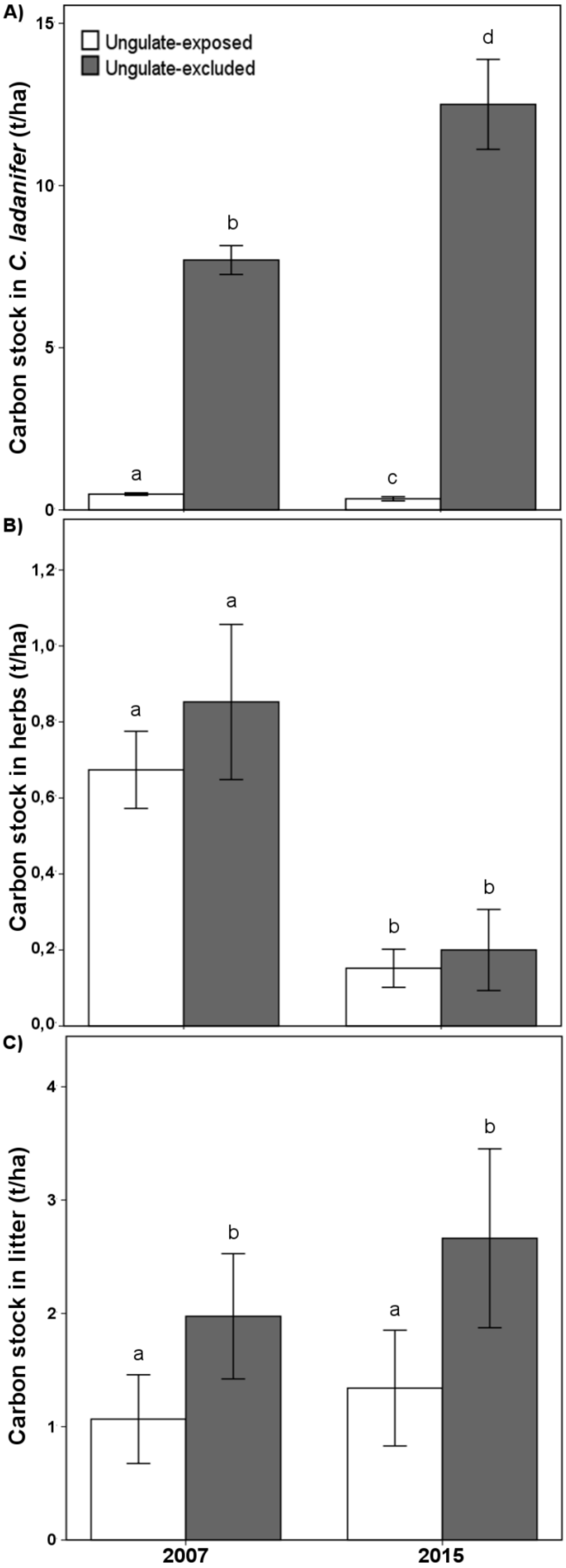

Figure 3: Aboveground C stocks in ungulate-excluded and ungulate-exposed plots in 2007 and 2015 (Mean ± sem): A) *Cistus ladanifer*, B) herbs and C) litter. Different letters indicate significantly (GLMM, $P < 0.05$) different values between treatments. Note that the scale range differs between graphs.

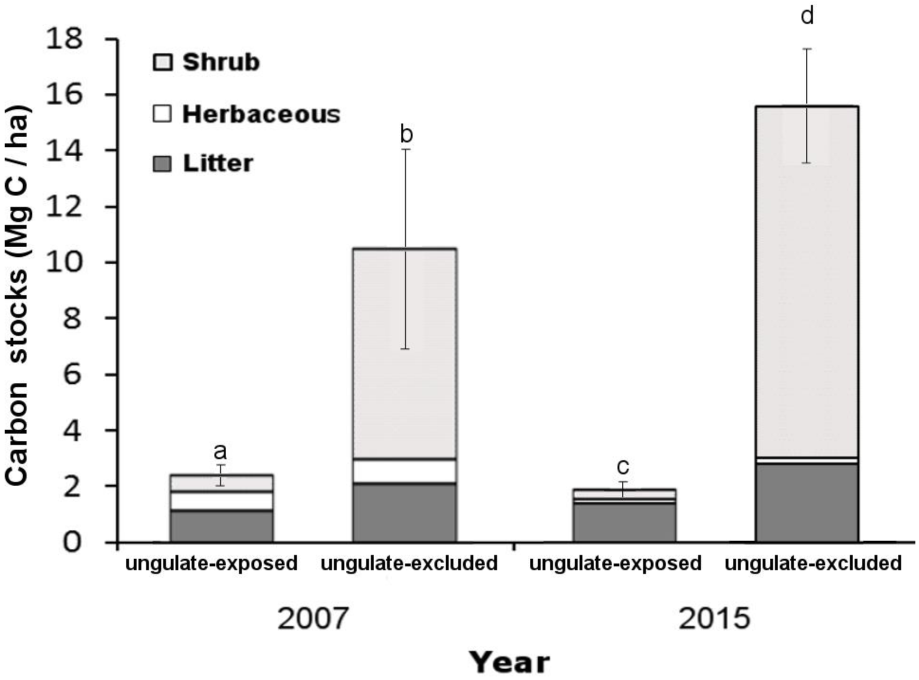

Figure 4. Aboveground C stocks of shrubs, herbs and litter in ungulate-exposed and ungulate-excluded plots, in 2007 and 2015. (Mean value ± sem). Different letters indicate significantly different (GLMM, $P < 0.05$) values between treatments) for total C stocks.

Effects of ungulates on fuel loads

Ungulate exclusion altered fuel load characteristics in a number of ways (Fig. 5). Live woody fuel load of *C. ladanifer* in ungulate-excluded plots in 2015 was higher than in control plots ($F_{2,142} = 19.35$, $P < 0.001$) as well as 1-h ($F_{2,142} = 26.43$, $P < 0.001$), 10-h ($F_{2,142} = 21.82$, $P < 0.001$) and 100-h ($F_{2,142} = 20.67$, $P < 0.001$) fuel loads. Indeed, shrub fuel load was 27-fold greater in ungulate-excluded than in the ungulate-exposed plots.

In contrast, browsing exclusion did not affect significantly herb fuel load (i.e. 1-h dead fuel load) or litter 10-h fuel loads (mean ± sem: 0.40 ± 0.10 vs. 0.30 ± 0.05 t/ha and 0.18 ± 0.08 vs. 0.19 ± 0.07 t/ha in ungulate-excluded and ungulate-exposed plots, respectively). Ungulate exclusion did have a significant effect on litter 1-h fuel load (Mann-Whitney U-test, U = 751, $P = 0.01$) which was twice as high in ungulate-excluded plots as in browsed plots (5.49 ± 0.78 vs. 2.66 ± 0.48 t/ha). No 100-h litter fuels were observed in ungulate-exposed plots.

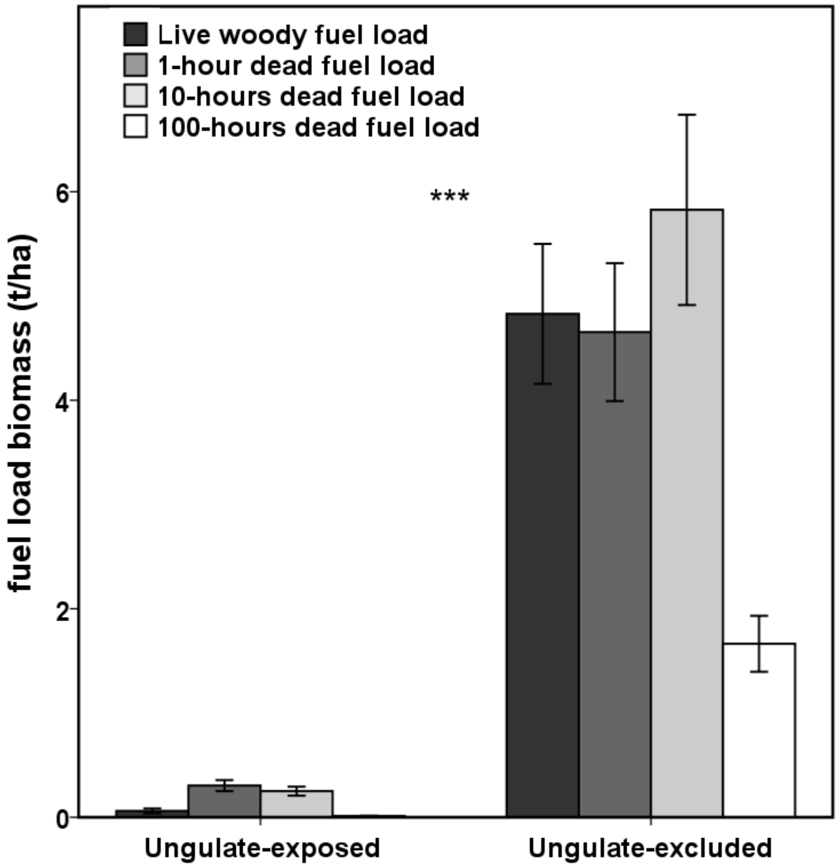

Figure 5. Fuel load of *C. ladanifer* by size class in ungulate-exposed and ungulate-excluded plots in 2015 (Mean ± sem). *** All differences in fuel load size classes between treatments were highly significant (GLMM, $P < 0.001$).

Fire behavior and effects on trees

Ungulate-exclusion led to a strong increase of estimated flame length, that exceeded 1.3 m, a critical threshold implying surface-to-crown fire transition, with crown torching, regardless of the wind speed considered (Fig. 6A). In browsed plots, flame length required wind speed > 20 km/h (Fig. 6A), which exceeds the maximum wind speed registered at our study site, to surpass the critical flame length threshold. In the ungulate-excluded plots surface rate of fire spread and fireline intensity also increased significantly by 2 and 18-fold, respectively as compared to browsed plots (Fig. 6B and 6C).

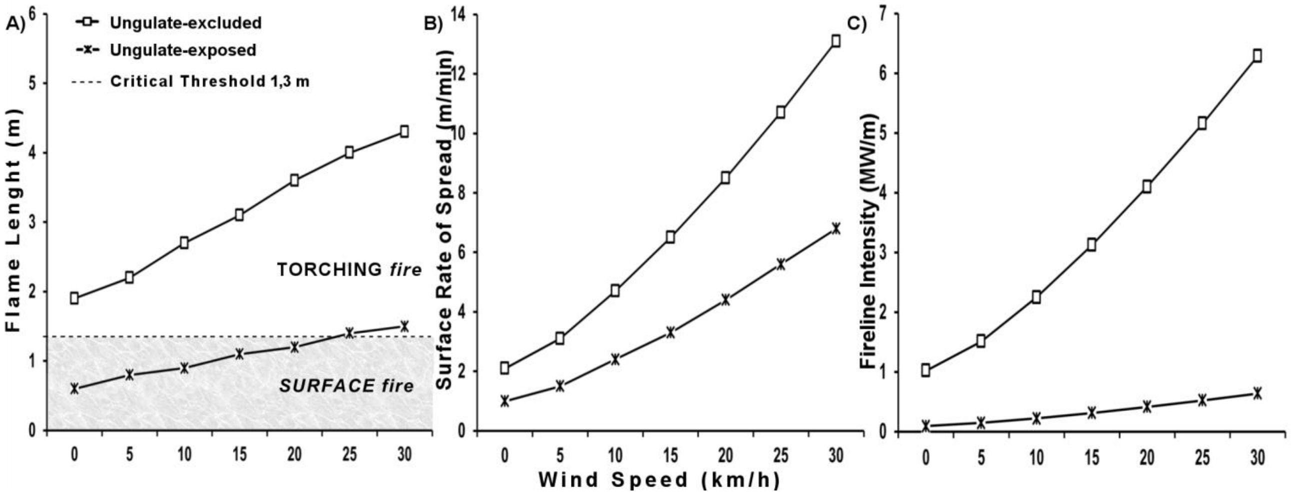

Figure 6. Fire behavior characteristics in ungulate-exposed and ungulate-excluded plots under increasing wind speed and constant slope of 5º as estimated with BehavePlus fire modeling system: A) Flame length, B) Surface rate of spread and C) Fireline intensity.

Oak tree mortality varied between 3 to 6% and 0 to 2%, in ungulate-excluded and browsed plots, respectively, as estimated from combining tree characteristics and models of post-fire tree responses with maximum char height on trees. The latter was estimated to be 100% in ungulate-excluded plots and 20% in control plots. Moreover, in the case of cork oak trees, which are harvested every 9 to 12 years for their bark (cork), for use in the wine industry, the probability of mortality decreased significantly with cork regrowth after harvesting (Figure 7). Our cork oak tree mortality estimates considered a bark thickness of 3.5 cm, which corresponds to the end of the bark growing cycle, immediately before cork harvest. For recently debarked trees, the probability of tree mortality would increase to 45% in ungulate-exposed plots and to 65% in ungulate-excluded plots (Fig. 7).

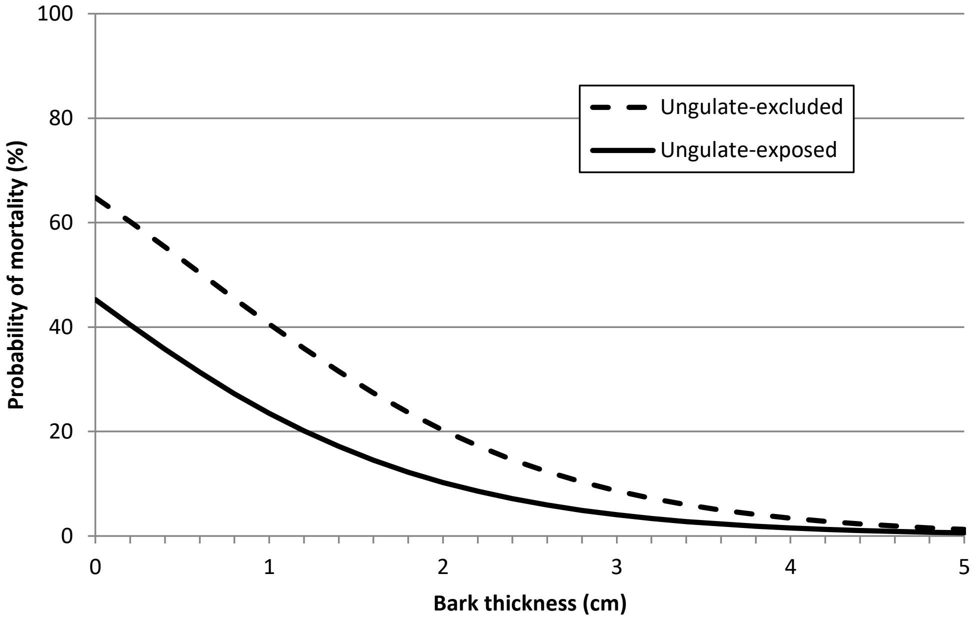

Figure 7. Probability of cork oak mortality (%) in relation to the bark thickness corresponding to the debarking cycle, based on Catry et al 2013 model of effects of fire on mortality of oak trees.

4.5 Discussion

Effects of ungulates on ecosystem structure and C stocks

Our study revealed substantial effects of ungulate browsing on population structure and biomass of *C. ladanifer*. Indeed, in 2015, at the end of our experiment, shrub density, height and C stocks in ungulate-exposed plots were reduced by 9-, 8- and 36-fold, respectively. Reductions of shrub biomass and thus C stocks, and plant density have been observed in other browsed ecosystems by different ungulate species in different regions as North America (e.g., mule *Odocoileus hemionus*, Huffman 2003; wapiti *Cervus canadensis*, pronghorn *Antilocapra americana* and mule deer *Odocoileus hemionus*, Singer and Renkin 1995) and Europe (e.g., reindeer *Rangifer tarandus*, Vowles 2016; roe *Capreolus capreolus* and red deer *Cervus elaphus*, (González Hernández and Silva-Pando 1996).Our results also showed significant reduction of litter C stocks by ungulates primarily attributable to the accumulation of shrub leaves and twigs in ungulate-excluded plots. Reduction of litter biomass by ungulates (Pajunen et al. 2012) can potentially and strongly decrease C stocks (Hughes et al. 2006).

In our sites, browsing had a strong negative effect on above-ground C stocks, affecting shrub and litter components, as observed elsewhere (e.g., Tanentzap and Coomes 2012). In Spain, in *C. ladanifer* encroached open woodlands of *Q. ilex* used by livestock, C stocks varied between 1.17 and 7.21 Mg C ha-1, which are within the range of our results. In our study we considered dead shrubs as C stocks components. Future research, however, should address how decomposition of such dead plant material affects estimates of long-term balance of C stocks (Keith et al. 2009). Although we did not include estimates of belowground C, *C. ladanifer* encroachment was shown to increase soil organic C by over 20% in oak woodlands in southern Portugal (Gomez-Rey et al. 2013). Moreover, the increased density and above-ground biomass of *C. ladanifer* in the ungulate-excluded plots likely reflected an increase in the C stocks associated to the roots of the shrubs. Root:shoot ratio for *C. ladanifer* was estimated as 0.56 ± 0.03 in a *Q. ilex* woodland in Spain (Ruiz-Peinado et al. 2013). However, this ratio can be lower in ungulate-exposed plots, as grazing may reduce belowground C stocks through negative effects on plant root biomass. For example, sheep grazing reduced by half the soil organic C in an eastern Australian semi-arid woodland of mulga (*Acacia aneura*) (Daryanto et al. 2013). Overall, our estimates of higher C stock in browsing-exclusion plots may have underestimated total C stocks of the system by not including changes in belowground. In our study, as found by other authors (Throop and Archer 2007, Zhou et al. 2007, Knapp 2008), shrub encroachment and browsing exclusion led to an increment of C inputs into the ecosystem, and ungulates were shown to apparently reduce aboveground C stocks, negatively affecting this regulating ecosystem service (sensu Millenium Ecosystem Assessment 2005). However, it is also noteworthy that aboveground C stocks of oak trees were not affected by ungulate exclusion.

Effects of ungulates on fire behavior

Our results emphasized a strong total fuel load reduction by ungulates, namely of fine fuels. Domestic and wild ungulates, such as deer, may be effectively used as management tools for reducing fuel load (Hobbs 2006, Davies et al. 2010, Ingram et al. 2013). Although effects may vary with animal type and population densities, ungulates (Ingram et al. 2013) can contribute to decrease fire hazard in Mediterranean regions. For example, goats were shown to disrupt the vertical continuity and accumulation of biomass of grasses and shrubs (Lovreglio et al. 2014). In Spain, goats reduced the volume of *C. ladanifer* by 45% over a period of 24 months (Mancilla-Leytón and Martín Vicente 2012) and decreased understory flammability by 25% after 4 years (Mancilla-Leytón et al. 2013). In Greece, goats reduced shrub understory height from 40% to 82% and decreased 1-h and 10-h fuel load by 33% and 58%, respectively (Tsiouvaras et al 1989).

Our fire modeling results showed a clear reduction of potential fire spread rate and intensity (50% and 90%, respectively) in the ungulate exposed plots. In particular, browsing exclusion increased the flame length and the likelihood of crown fire transition. Crown fires are severe fires that are more likely to induce tree mortality, in contrast to lower intensity surface fires (Agee and Skinner 2005). Numerous studies have shown a negative impact of high-intensity fire on tree survival (Williams et al. 1999, Hoffmann and Solbrig 2003, Ritchie et al. 2007). Severe crown fires can be hindered in areas browsed by ungulates, which may ultimately favor adult tree survival.

Our estimates indicated that tree mortality due to fire hazard can be 3-fold greater in browsing-excluded plots. In the case of cork oak trees, however, tree mortality will further increase in recently debarked trees (Catry et al. 2012), impacting tree and ecosystem C storage. Indirect positive effects of ungulates on adult tree survival is critical to maintain ecosystem C stocks if a fire occurs as oak trees are the major reservoir of aboveground C in these ecosystems. Paradoxically, in this study we show that ungulates, which are usually associated with negative effects on trees (e.g., Ramos et al. 2006, White 2012) may ultimately contribute to oak tree survival and the sustainability of the system.

Effects of land abandonment, with subsequent encroachment by flammable shrubs, and afforestation, associated with decreased forest management, are implicated in the fire regime changes observed in Mediterranean regions during the last decades (e.g. Fernandes et al. 2014, Pausas and Fernández-Muñoz 2011). Moreover, weather extremes which are now more common (Trigo et al. 2006, Rego and Silva 2014) also contribute to increase the frequency and intensity of wildfires (IPCC 2007). Wildfire prevention is thus a crucial ecosystem service in Mediterranean regions. In Iberian Peninsula, as in other Mediterranean regions, where severe wildfires are increasing in frequency but ungulate hunting is also widespread and ungulates maintained at relatively high population densities, ungulates may be used as an effective fuel treatment option whilst also contributing to maintain long-term C stocks.

4.6 Conclusions

Based on our 14-year experiment we found that ungulate browsing mediated C stocks and fire-hazard mitigation ecosystem service trade-offs in a Mediterranean oak woodland. Through effects on plant biomass and litter, ungulates may prevent sudden C losses resulting from severe wildfires. Recent expansion of ungulate populations in the Northern hemisphere, with negative effects on tree regeneration and damage to adult trees (Ramos et al. 2006, Bugalho et al. 2013) and loss of understory C stocks need to be considered against significant reductions of fire-hazard in fire-prone ecosystems. Within the context of climate change scenarios, with predicted increase frequency of severe wildfires in Mediterranean ecosystems in particular, research on effects of different ungulate communities on ecosystem services trade-offs is clearly needed in the future.

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4.7 Supporting information

Appendix 1. Regression equations for *Quercus ilex* ssp. r*otundifolia* and *Quercus suber* with W: weight biomass with diameter smaller than 6 mm (kg) and D: diameter at breast height (m). (F and *P*-value for Anova test for regression model)

	Equation	R^2	F	P-value
Quercus rotundifolia	$W = 510{,}842\ D^{2{,}002}$	0.99	1922.14	<0.001
Quercus suber	$W = 32{,}527\ D^{1{,}285}$	0.684	56.41	<0.001

Appendix 2. Biometric parameters for Q. suber and Q. rotundifolia between ungulate-excluded and ungulate-exposed plots. Mann-Whitney U test was used when normal distribution and homogeneity of variance were not verified

		Holm oak	Cork oak	Statistics
Canopy height (m)	ungulate-exposed	7.21 ± 0.41	8.12 ± 0.41	t-test, t(20) = -1.044, *P* = 0.740
	ungulate-excluded	7.51 ± 0.38	8.77 ± 0.44	t-test, t(25) = -2,109, *P* = 0.045
	Statistics	t-test, t(19) = -0.249, P = 0.806	t-test, t(26) = -1.243, *P* = 0.225	
Canopy base height (m)	ungulate-exposed	2.22 ± 0.06	2.34 ± 0.07	t-test, t(20) = -1.991, *P* = 0.060
	ungulate-excluded	2.13 ± 0.16	2.40 ± 0.28	Welch test, F = 0,733, *P* = 0,401
	Statistics	Welch test, F = 0,065, P = 0,802	Mann-Whistney U test, U = 93,5 *P* = 0,856	
Diameter at breast height (cm)	ungulate-exposed	38.70 ± 3.01	43.17 ± 1.89	t-test, t(20) = -1.044, *P* = 0.309
	ungulate-excluded	38.98 ± 2.57	46.86 ± 2.92	t-test, t(25) = -1,992, *P* = 0.057
	Statistics	t-test, t(19) = -0.086, P = 0.932	t-test, t(26) = -1.117, *P* = 0.274	
biomass Ø < 6 mm	ungulate-exposed	86.48 ± 11.89	10.98 ± 0.95	Mann-Whistney U test, U = 0,000 *P* < 0,001
	ungulate-excluded	77.78 ± 10.63	12.77 ± 1.19	Mann-Whistney U test, U = 0,000 *P* < 0,001
	Statistics	t-test, t(19) = 0.543, P = 0.594	Mann-Whistney U test, U = 123,0 *P* = 0.254	

Appendix 3. Biometric parameters for the whole tree layer in the ungulate-excluded and ungulate-exposed plots. Mann-Whitney U test was used when normal distribution and homogeneity of variance were not verified.

	Ungulate-excluded	Ungulate-exposed	Statistics
Canopy cover (%)	59.89 ± 2.60	59.14 ± 6.56	t-test, t(6) = 0.107, *P* = 0.918
Canopy height (m)	8.21 ± 0.32	7.75 ± 0.30	t-test, t(47) = 1.044, *P* = 0.302
Canopy base height (m)	2.28 ± 0.17	2.29 ± 0.05	Mann-Whitney U test U = 266.00, *P* = 0.532
Canopy bulk density (kg/m3)	0.13 ± 0.01	0.09 ± 0.03	t-test, t(6) = -1.407, *P* = 0.209
Diameter at breast height (cm)	43.34 ± 2.10	41.34 ± 1.69	t-test, t(47) = -0.711, *P* = 0.481

CHAPTER 5

COMBINED EFFECTS OF DEER BROWSING AND DROUGHT ON *C. LADANIFER* MORTALITY AND FIRE HAZARD

MANUSCRIPT: Lecomte X., Bugalho M.N, Catry F.X., Fernandes P.M., Caldeira M.C.. 2017. Drought and shrub encroachment increase fire hazard in a Mediterranean evergreen oak woodland ecosystem

5.1 Abstract

Climate change and extreme events, such as drought, can affect ecosystem functioning and often interact with other global change factors such as shrub encroachment and wildfire. Shrub encroachment is a global world-wide phenomenon, which high flammability ranks them among the most fire-prone ecosystems. In this study, we explored the combined effects of drought and shrub encroachment by *Cistus ladanifer*, on the susceptibility to fire of a Mediterranean oak woodland. We especially compared, through a long-term ungulate exclusion experiment, changes in spatial structure of *C. ladanifer* population, shrub recruitment, and mortality after an extreme drought year (2011-2012). We also modeled and compared fire behavior of the shrub encroached and browsed stands in two scenarios: 1) after the extreme drought year and, 2) in a scenario of non-drought. In the shrub encroached stands, shrub biomass and fuel accumulation were 36-fold and 25-fold higher, in average, than in the ungulate browsed plots, increasing drastically modeled fire parameters. Drought induced dramatic shrub mortality, which was of 60% of total shrub population at the end of the experiment in the ungulate-excluded plots. Combined effect of browsing and drought increased mortality to 84% in the ungulate-exposed plots. Shrub recruitment was, in average, for the two studied years after drought (i.e. 2013 and 2015), 80-fold higher in the ungulate-excluded plots than in ungulate-exposed plots. By decreasing live to dead ratio of plant biomass, drought increased, in both treatments, flame length (i.e. by 17% in average), surface rate of spread (i.e. 14%) and intensity (i.e. 45%) of wildfire which may increase potential damages to adult oak trees. However, although a reduction of live to dead ratio was observed in ungulate-exposed plots, browsing dampened the negative effects of drought and the modeled fire parameters. These results emphasized the strong negative combined effects of shrub encroachment and drought on fire hazard and increased the need to consider management strategies, as ungulate browsing, to mitigate efficiently fire hazard.

Keywords: above-ground biomass, shrub density, fire behavior, ungulates, drought-induced mortality, fuel load, BehavePlus.

5.2 Introduction

Extreme droughts are expected to increase in frequency and severity in the context of ongoing climatic changes in the Mediterranean regions (IPCC 2014). Droughts can affect ecosystem functioning by decreasing soil water availability, plant transpiration and productivity (Peñuelas et al. 2007, Michra and Singh et al. 2010, Caldeira et al. 2015, Liu et al. 2015), and ultimately change species composition and ecosystem structure through increased plant mortality (van Mantgem et al. 2009, Allen et al. 2010, Camarero et al. 2015). However, climate changes often interact with other global change factors, such as shrub encroachment or wildfires, although the impacts of their combination are seldom understood (Doblas-Miranda et al. 2017). Shrub encroachment, the increase in shrub density and biomass, is occurring in a widespread manner in dryland grasslands and savanna-type of ecosystems worldwide (Van Auken 2000, O'Connor et al. 2014, Archer at al. 2017). Shrublands provide numerous ecosystem services such as prevention of soil erosion (Boeken and Orenstein 2001) or carbon sequestration (Pacala et al. 2001, Janssens et al. 2003, Piao et al. 2009), but can also decrease water availability for trees by increased competition (Caldeira et al. 2015) or increase fire hazard (Lecomte et al 2017). Shrublands and shrub encroached ecosystems, however are among the most fire-prone ecosystems, (Syphard et al. 2007, Moritz et al. 2014, Rego and Silva 2014), particularly in Mediterranean regions. In these regions during the dry and hot late spring and summer, fire hazard depends much on the structural characteristics of the fuel vegetation (Fernandes 2009). A dense spatial structure and connectivity as well as a frequently high flammability may strongly favor propagation, high flame length and fire line intensity in shrublands (Fernandes et al. 2000, De Luis et al. 2004, Pausas and Bradstock 2007). Additionally, susceptibility of shrublands to fire may increase with drought. First, by decreasing moisture content, drought may increase plant flammability (Pellizzaro et al. 2007, Dennison and Moritz 2009), second because fire hazard depends on the availability and amount of live fine fuel and dead fractions (Baeza et al. 2006, Baeza et al. 2011) which may increase with plant mortality induced by drought. Understanding how extreme droughts combine with shrub encroachment potentially affecting ecosystem structure and susceptibility to fire is of increasing interest under the ongoing climatic changes.

In this study, through a deer exclusion experiment, we addressed the combined effects of drought and shrub encroachment on the susceptibility to fire of a Mediterranean open oak woodland. Specifically, we compared the vertical and horizontal structure of *C. ladanifer* population as well as shrub recruitment and adult survival after an extreme drought year (2011-2012) in plots continuously browsed by deer (red deer, *Cervus elaphus* and fallow deer, *Dama dama*) (control) or where browsing was excluded (shrub encroached) during the last 14 years. In addition, we modeled the fire hazard of the two treatments (no browsed and control) after the extreme drought event and compared it to a modelled no drought scenario. We hypothesized that drought: 1) alters the structure and biomass of *C. ladanifer* stands in both browsed and deer- excluded plots; 2) increases fire hazard particularly in the shrub encroached stands by increasing *C. ladanifer* mortality during the extreme drought year with a subsequent recruitment of young shrub plants that contributes to total fine fuel load.

5.3 Methods

Study site and sampling design

This research was conducted in Tapada Real de Vila Viçosa, an 900 ha enclosed estate in southeast Portugal (38°49'N, 07°24'W). The study area is characterized by a savannah-type ecosystem of a mixed open tree layer of evergreen holm oak (*Quercus ilex* ssp. *rotundifolia* Lam) and cork oak (*Quercus suber*) with an understory dominated by the semi-deciduous shrub gum cistus (*Cistus ladanifer*) and annual grasslands composed by grasses (e.g. *Bromus madritensis*, *Gaudinia fragilis*), forbs (e.g. *Andryala integrifolia*, *Leontodon taraxacoides*) and legumes (e.g. *Vicia disperma*, Ornithopus compressus). *C. ladanifer* is common to the Western Mediterranean area, (Guzmán and Vargas 2009). the terpene-derived resin that impregnates leaves and branches makes *C. ladanifer* individuals easily flammable (Núnez-Regueira et al. 2005). These compounds may also indirectly delay litter decomposition and accelerate fine fuel accumulation (Horner et al. 1988). *C. ladanifer* stands are characterized by a low fuel moisture content during the summer which was shown to be highly correlated with fire occurrence and spread (Chuvieco et al. 2009). *C. ladanifer* is also an important source of forage for the browsers present in the estate (Bugalho and Milne 2003, Lecomte et al. 2016, Lecomte et al. 2017). There was a population of red (*Cervus elaphus*) and fallow (*Dama dama*) deer, with a density of 0.35 and 0.1 deer per ha, respectively, at the beginning of the study (Bugalho et al. 2011).

Mean annual precipitation in the study site is 585.3 mm mostly distributed between October and May and mean temperatures reach 31.1ºC in July and 5.8ºC in January (mean annual temperatures of 15.9ºC) (Évora meteorological station, 1981-2010, htpp://www.ipma.pt, accessed in June 2017). The year of 2011-2012 was an extremely dry year in the study area in agreement with the rest of Southwest Europe (Trigo et al. 2013). In the region of our study area, total precipitation of the hydrological year (i.e. from October 2011 to September 2012) was only 55% of the long-term mean precipitation (Fig. 1). This drought period was associated with a high autumn precipitation deficit (i.e. 36% lower than long-term mean precipitation for the same period), which was extreme in winter (i.e. 80% lower) and moderate in spring (i.e. 24% lower).

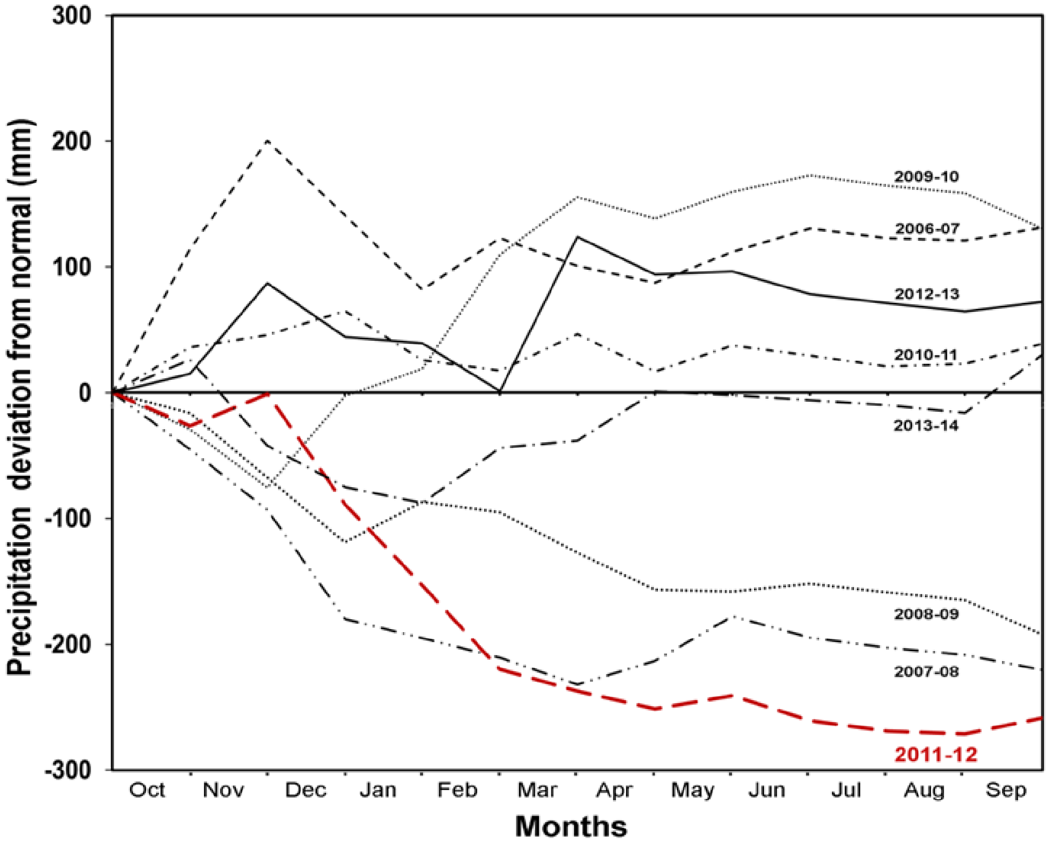

Figure 1. Accumulated precipitation deviations from climatic normal (1981-2010) during the hydrological year (October-September) for Évora (Mitra station, 38°31'N. 8°01'W: http://www.cge.uevora.pt, September 2017)

Experimental design

Five blocks of paired ungulate-excluded and ungulate-exposed plots of 25 m × 25 m were established in July 2001 in homogeneous areas of grassland. These areas had been previously mechanically cleared of *C. ladanifer*, as part of the common management practices in the estate. Ungulate exclusion plots were established with a fence of 2.20 m height. Each open and fenced plot held similar proportions of *Q. suber* and *Q. rotundifolia* trees (i.e. 2 or 3 trees from each species) and was divided in 40 sub-plots of 2 m x 4 m each. Eighteen sub-plots were randomly selected to conduct the experiment. One open plot was indivertibly ploughed and lost in July 2004, hence we just use data from the remaining 4 pairs of plots

Shrub measurements

C. ladanifer population density was estimated in March 2007, 2013 and 2015, by counting all living and dead individual shrubs occurring in the 18 sub-plots. In 2015, the maximum height of *C. ladanifer* stands was measured in 10 randomly selected subplots (i.e. total of 40 *C. ladanifer* individuals in ungulate-excluded and ungulate-exposed plots), by measuring the tallest *C. ladanifer* individual in that plot. In addition, we measured basal trunk diameter in all counted shrubs.

In 2015, we also estimated the stand shrub aboveground biomass through allometric equations relating *C. ladanifer* basal trunk diameter and biomass. To achieve this we randomly selected 6 to 10 living individuals of *C. ladanifer* per plot (total of 30 shrubs per treatment) and distributed per trunk diameter class (i.e. 10 mm diameter classes between 6 to 86 mm). Selected shrubs were cut to ground level, oven-dried at 80ºC to constant mass and weighed. Branch structure and volume were comparable for both dead and live individuals; we then estimated stand dead biomass through allometric equations between trunk diameter and biomass, deducting leaves biomass. Shrubs with a basal trunk diameter smaller than 10 mm in 2015 were considered as young recruited shrubs.

C. ladanifer fuel load characteristics were also evaluated in 2015, in both treatments, to model fire behaviour (see fire modelling section). Shrub biomass was separated into dead and live components and, as ignition of woody fuel depends of the stem diameter, all component were separated by diameter size classes: live woody fuel load (i.e. live components including foliage and stems with diameter < 0.64 cm), 1-h dead fuel load (i.e. stems < 0.64 cm), 10-h dead fuel load (i.e. stems with diameter between 0.64 and 2.54 cm) and 100-h dead fuel load (i.e. stems > 2.54 cm).

Herb and litter biomass

Herb biomass was estimated in June 2015, coinciding with the peak of herb biomass production, in 4 randomly chosen sub-plots. Litter biomass was assessed in 8 randomly chosen sub-plots, distributed equally beneath the tree canopy and in the open area. All above-ground material was clipped to ground level within a 50 cm x 25 cm quadrat. All material was separated by the same diameter class described above (i.e. 1-h, 10-h and 100-h fuel load), oven-dried at 60 ºC to constant mass and weighed.

Fire modelling

Fire behaviour modelling in the ungulate-excluded and ungulate-exposed plots were performed using BehavePlus 5.0.5 fire modelling software (Andrews 2009, Andrews 2014), which allowed to predict potential wildfires characteristics. We used input data on vegetation fuel characteristics of shrubs, herbs and litter, weather and topography (Andrews 2014). Shrub cover and heights and total dead fuel load separated by timelag classes (i.e. 1-h, 10-h and 100-h dead fuel load) and live woody fuel load where used. Herb layer, which senesced and died during summer was added in the 1-h dead fuel load. Fuels moisture values were considered as, 4% for 1-h, 5% for 10-h, 6% for dead 100-h and 75% for live woody fuel load. Foliar moisture was considered as 80 % (Chuvieco et al. 2009, Fernandes 2009). Fuel bed height was corrected using an empirical model developed for shrublands (Burgan and Rothermel 1984). SURFACE, CROWN and SCORCH Modules were used to predict wildfire behaviour in the understory vegetation and the potential impacts of the tree layer. Simulation were performed for temperature of 31 ºC, slope of 5º (range value of our plots: 1.4º to 7.5º, mean of 4.58 ± 0.55 º) and for a range of wind speed from 0 to 30 km.h-1. Flame length (m), surface rate of spread (m/min) and fireline intensity (MW/m) output were studied.

To explore the combined effect of drought and ungulate exclusion on fire behaviour, we did the same modelling exercise for fire behaviour considering that all shrubs were alive (no drought) and no contribution of the leaves of the dead shrubs to the litter fuel load.

Statistical analysis

Data on *C. ladanifer* density, mortality and young recruited shrubs were analysed fitting generalized linear mixed models. Due to high number of zero values in *C. ladanifer* count for shrub density and young recruited shrubs emergence, we used a negative binomial error and log-link function for shrub density. Binomial and log-link function was used for shrub mortality. Ungulate exclusion, year and interaction between ungulate exclusion and year were specified in the models as fixed effect and subplot, plot and replicate (nested within plot) were included as random factor. For *C. ladanifer* biomass, litter and herbs biomasses and fuel loads from 2015, normal distribution and identity-link function was used. All statistical analyses were carried out by using of the SPSS® software package (PASW Statistics, v. 23.0.0.0, 2015).

5.4 Results

Effects of shrub encroachment and drought on the structure of C. ladanifer populations.

Shrub encroachment had a strong effect on the structure of *C. ladanifer* stands with total shrub density being, on average over 3-fold higher in the ungulate-excluded plot than in the ungulate-exposed plots ($F_{1,426}$ = 5.91, $P = 0.015$) (Fig. 2). Year did not have a significant effect as main factor ($F_{2,426}$ = 0.37, $P = 0.694$) and there were no significant interactions between ungulate exclusion and year ($F_{2,426}$ =0.31, $P = 0.733$), indicating consistency of the effect on shrub encroachment over the years.

Drought had a strong effect on shrub encroachment. Whereas no dead shrubs were observed in both treatments in 2007, in 2013, after the extreme drought year, shrub mortality in the ungulate-excluded plots was 10% of the shrub population (Fig. 2). This proportion increased significantly along time reaching 60% of the population in 2015 ($F_{2,111}$ = 12.60, $P < 0.001$). Our experiment also showed an interactive effect between drought and browsing on shrub mortality with higher proportion of dead shrubs in the ungulate-exposed plots compared to the ungulate-excluded plots ($F_{2,193}$ = 23.40, $P < 0.001$).

Browsing (control plots) increased the proportion of dead shrub by over 670% in 2013 (70% of dead individuals in 2013) and by 40% in 2015 (84% of dead individuals in 2015), comparing to the ungulate-excluded plots. Mortality affected all *C. ladanifer* trunk diameter classes: [0-10 mm] and [30-40 mm] diameter classes were the most affected in the ungulate-exposed plots with 69% and 66% of mortality, respectively, while that were [20-30 mm] and [30-40 mm] diameter classes in the ungulate-excluded plots with 90% and 87% of mortality, respectively.

Living shrub density decreased between 2007 and 2015 in the browsed plots (i.e. by 65% between 2007 and 2013 and by 47% between 2013 and 2015) ($F_{3,213} = 15.95$, $P < 0.001$), while in the ungulate-excluded plots, live shrub density increased by 160% between 2007 and 2013 ($F_{2,142} = 34.71$, $P < 0.001$) but decreased 50% between 2013 and 2015 ($F_{2,142} = 86.26$, $P < 0.001$).

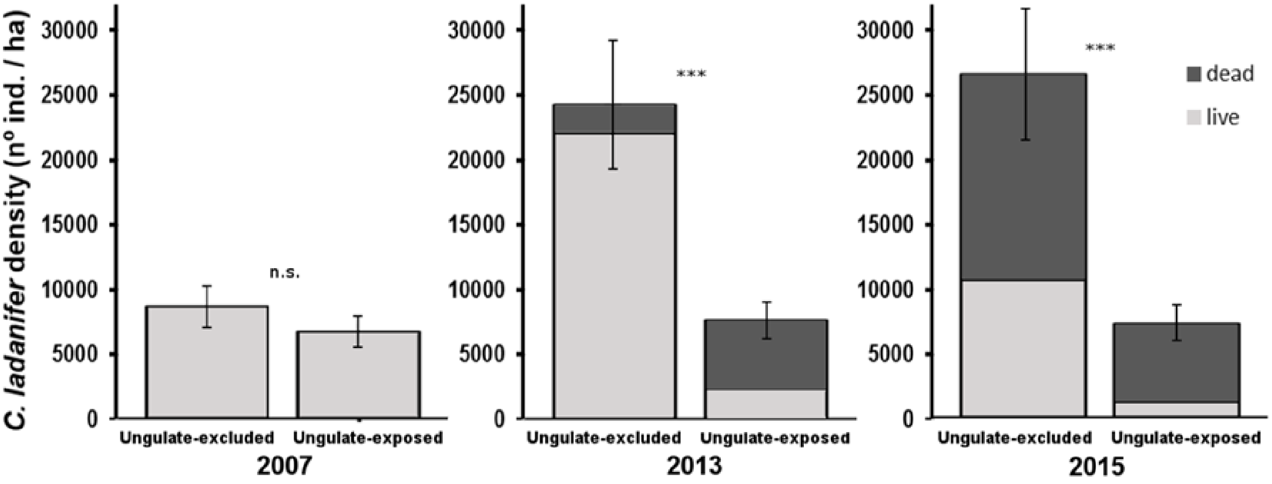

Figure 2. Number of *C. ladanifer* living and dead individuals per ha in ungulate-excluded and ungulate-exposed (i.e. control) plots in 2007, 2013 and 2015. (mean ± sem for total number of individuals), *** indicate significantly different values between treatments ($P < 0.001$)

While no substantial seedling emergence neither young shrubs were observed in 2007 (personal observation) there was a strong seedling recruitment in the ungulate-excluded plots in 2013, a year after the extreme drought occurred; Indeed, in 2013, live young shrubs amounted to 48% of the total living population (i.e. living adult and young individuals) and no dead young individuals were observed (Table 1). In 2015, living young individuals amounted for 28% of the total living population but 67% of young individuals were found dead within the young population.

Browsing affected seedling emergence negatively reducing the number of young individuals by 98% in 2013 and by 97% in 2015 ($F_{1,284} = 14.00$, $P < 0.001$) (Table 1). There were no interactions between year and treatment (2013 and 2015, $P = 0.920$) as mean factor, that is browsing had a consistent and negative effect on young individuals over time. No dead young individuals were found in the browsed plots either in 2013 or 2015.

Table 1. Young live and dead *C. ladanifer* individuals (n/ha) observed in 2013 and 2015 in the ungulate-exposed (i.e. shrub management treatment) and ungulate-excluded plots. (mean ± sem). Letters indicate significant differences (*P* < 0.05) between treatments.

	2013		2015	
	live	dead	live	dead
Ungulate-excluded	10625 ± 4540 ^{a}	0	2986 ± 659 ^{c}	6059 ± 3854
Ungulate-exposed	191 ± 77 ^{b}	0	87 ± 45 ^{b}	0

After 14 years (i.e. 2015), maximum height of *C. ladanifer* stands strongly increased with shrub encroachment, which was over 7-fold taller in the ungulate-excluded plots (2.88 ± 0.10 m), as compared with the ungulate browsing plots (0.38 ± 0.02 m).

Effects of shrub encroachment and drought on C. ladanifer aboveground biomass

C. ladanifer biomass dramatically increased in the shrub encroached plots (24.56 ± 2.73 t/ha and 0.67 ± 0.13 t/ha in the ungulate-excluded and ungulate-exposed plots respectively; $F_{2,142} = 38.89$, $P < 0.001$) (Fig. 3). Both dead biomass and live biomass were significantly higher in the ungulate-excluded plots compared to ungulate-exposed plots ($F_{2,142} = 20.46$, $P < 0.001$ and $F_{2,142} = 19.38$, $P < 0.001$ respectively). However, the ratio of live to dead individuals differed between treatments. In the ungulate-excluded plots dead biomass accounted for 46% of total biomass (although not differing from live biomass; $F_{1,142} = 0.44$, $P = 0.51$), with a live-to-dead ratio of 1.17. In the ungulate-exposed plots, dead biomass reached 83% of the total biomass and was significantly higher than live biomass ($F_{1,142} = 32.79$, $P < 0.001$) with a live-to-dead ratio of 0.20, attesting a strong effect of ungulate browsing on *C. ladanifer* mortality.

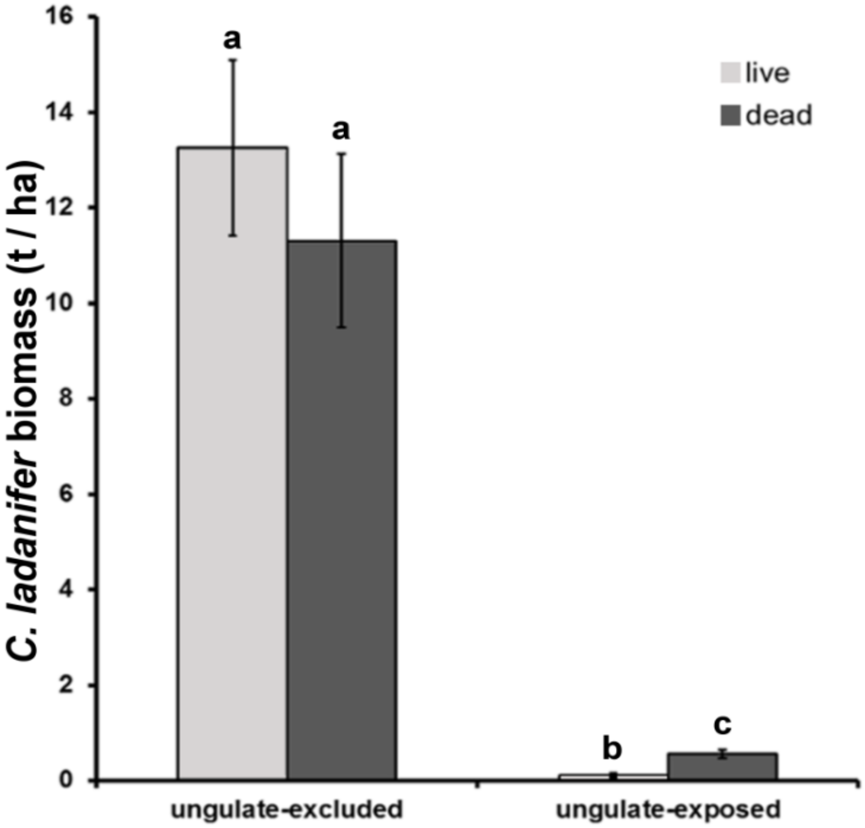

Figure 3. *C. ladanifer* dead and live plant material in 2015 in ungulate-excluded and ungulate exposed plots. (mean ± sem), *** indicate significantly different values between treatments ($P < 0.001$).

Additionally, herb biomass was not affected by shrub encroachment (0.40 ± 0.10 t/ha and 0.30 ± 0.47 t/ha in the ungulate-excluded and ungulate-exposed plots respectively; $P = 0.494$) but litter biomass significantly increased by 50% (5.67 ± 0.82 t/ha and 2.85 ± 0.53 t/ha in the ungulate-excluded and browsed plots respectively; $F_{1,62} = 8.23$, $P = 0.006$).

Effects of shrub encroachment and drought on fuel loads

Fuel load strongly increased in shrub encroached plots (Fig. 4). Total fuel load of *C. ladanifer* population was, on average 25-fold higher in ungulate-excluded plots than in ungulate-exposed plots regardless the scenario (i.e. 1) with and 2) without drought) ($F_{2,286} = 39.18$, $P < 0.001$). All classes of fuel load were significantly higher in the ungulate-excluded plots than ungulate-exposed plots (live woody, $F_{2,286} = 34.67$, $P < 0.001$; 1-h, $F_{2,286} = 30.82$, $P < 0.001$; 10-h, $F_{2,286} = 23.11$, $P < 0.001$ and 100-h, $F_{2,286} = 20.67$, $P < 0.001$).

Drought strongly decreased the ratio of *C. ladanifer* live to dead fine fuels by over 85% and 97% in ungulate-excluded (i.e. 7.40 and 1.04 for no drought and drought scenario, respectively, $F_{2,109} = 98.28$, $P < 0.001$) and ungulate-exposed plots (i.e. 8.25 and 0.20 for no drought and drought scenario, respectively, $F_{2,82} = 1479.51$, $P < 0.001$), respectively (Fig. 4). In the absence of ungulates, drought reduced live woody fuel load component by 2-fold ($F_{2,142} = 33.55$, $P < 0.001$) and increased 1-h (nearly 4-fold; $F_{2,142} = 27.76$, $P < 0.001$) and 10-h (over 16-fold; $F_{2,142} = 22.04$, $P < 0.001$) fuel loads, simultaneously generating 100-h fuel load ($F_{2,142} = 20.67$, $P < 0.001$) (Fig. 4: a) and b)). In the ungulate-exposed plots, drought reduced live woody fuel load component by nearly 7 fold ($F_{2,142} = 27.17$, $P < 0.001$), increased 1-h (nearly 6-fold; ($F_{2,142} = 32.77$, $P < 0.001$)) and 10-h (60-fold; ($F_{2,142} = 34.32$, $P < 0.001$)) fuel load and also produced 100-h fuel load ($F_{2,142} = 34.37$, $P < 0.001$) (Fig. 4: c) and d)). However, browsing dampened the negative effect of drought. Indeed, drought increased total fuel load by 58% in ungulate-excluded plot but only 32% in ungulate-exposed plots.

Fire modeling

In the shrub encroached plots flame length increased dramatically, being on average nearly 3-fold taller in the ungulate-exposed plots than in ungulate-excluded plots (Fig. 5). Flame length exceeded the critical threshold of 1.3 m, implying a surface to oak tree crown fire transition, whatever the wind speed considered and for both drought and non-drought scenarios. The surface rate of spread and fireline intensity were, on average, 2-fold and 10-fold higher in the ungulate-excluded plot compared to ungulate-excluded plots, respectively (Fig. 5).

Drought also had a strong effect on fire behavior. In the ungulate-excluded plots, drought affected flame length, surface rate of spread and fireline intensity in average by 17%, 14% and 45%, respectively, as compared to no drought scenario.

In the ungulate-exposed plots, flame length was over 5% higher in the scenario of drought than in the non-drought and a wind speed above 20 km/h will be required for inducing surface-to-crown fire transition (Fig. 5A). Drought also increased surface rate of spread and fireline intensity by 18% and 16%, respectively. However ungulate browsing seemed to dampen the negative effect of drought, which increased modeled flame length and fireline intensity to a lesser extent, as compared to the ungulate-exclude plots.

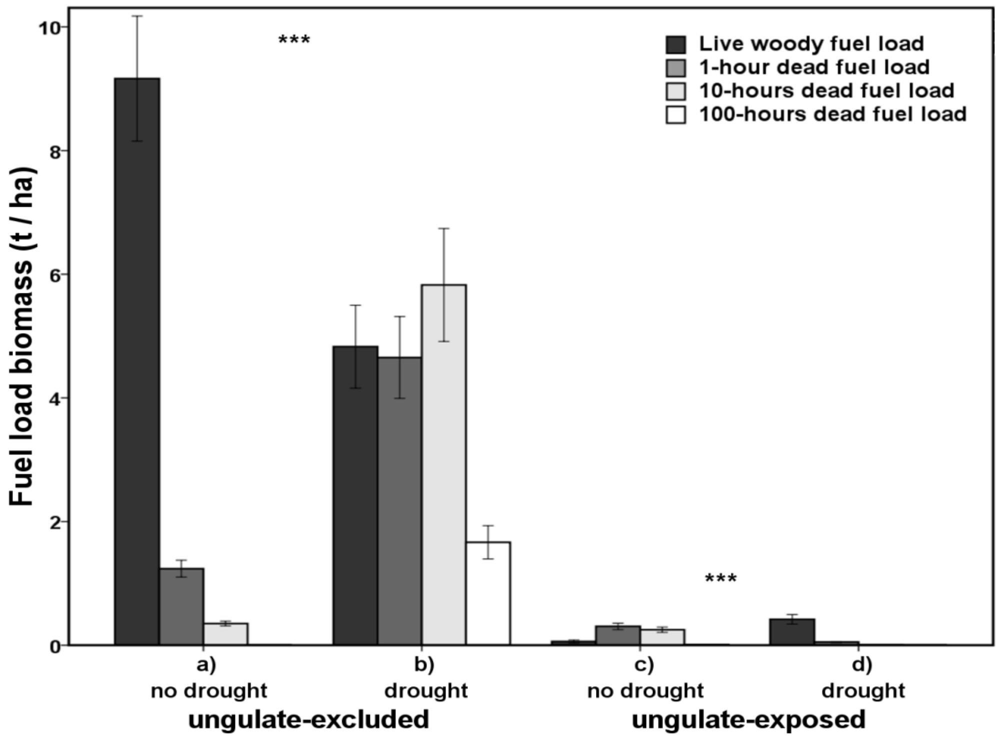

Figure 4. Fuel load of *C. ladanifer* by size classes in ungulate-exposed and ungulate-excluded plots in 2015 for different scenarios: a) and c) fuel load simulation without drought and b)and d) fuel load simulation considering drought. (Mean ± sem), *** indicated significant differences for both treatments between the scenarios with or without drought. Drought significantly affected all components of fuel load.

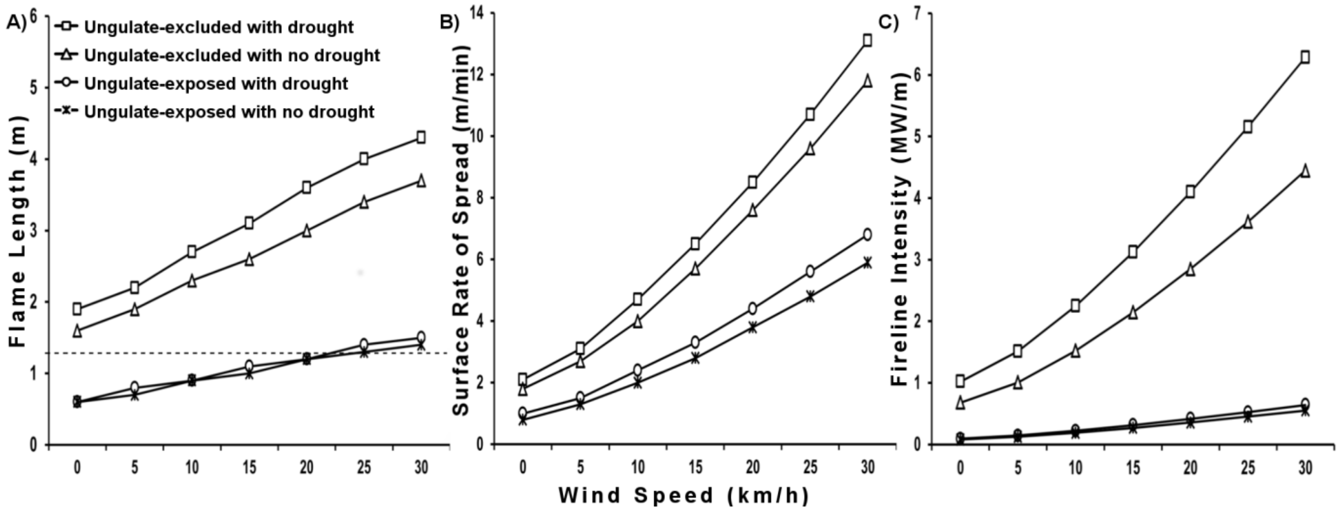

Figure 5. A) Flame length (m), B) Surface rate of spread (m/min) and C) Fireline intensity (MW/m) for 2 scenarios (i.e. drought event and no drought event) in ungulate-exposed and ungulate-excluded plots under increasing wind speed and constant slope of 5º. Dotted line in figure A) is the critical threshold of 1.3 m flame length implying surface-to-crown fire transition.

5.5 Discussion

Our results showed that shrub encroachment had a significant effect on fire behavior. Propagation of wildfires is strongly dependent on the vertical (i.e. relative height of vegetation fuel) and horizontal (i.e. density of the vegetation fuel) structure of the woody vegetation. The better the vertical and horizontal continuity of the woody vegetation the easiest is the propagation of the wildfire (Fernandes 2001, De Luis et al. 2004). Shrub encroachment is known to increase spatial structure and fuel load of understory, which, consequently, increase fire hazard and behavior (Fernandes 2009, Porto et al. 2013, Wang et al. 2016). Thus, the increase of shrub density and height and also biomass and fine fuel load in our shrub-invaded plots (i.e. ungulate-excluded plots) dramatically increased surface rate of spread, flame length and fireline intensity as shown in our previous study (Lecomte at al. 2017 in prep.). By the contrary, fuel load and fire hazard were mitigated in the ungulate-exposed plots through browsing as has been observed in other studies with domesticated animals such as goats and sheep (Campbell and Taylor 2006, Nader et al. 2007, Ingram et al. 2013, Lovreglio et al. 2014) and wildlife (Endress et al. 2012, Kimuyu et al 2014). The long term browsing by deer decreased all fuel load classes significantly reducing flame length, surface rate of spread and fireline intensity.

However, drought by inducing high shrub mortality, which reduced by 60% (in 2015) the density of living shrub in the ungulate-excluded plots, simultaneously increased the proportion of dead biomass and total fuel load by 58%, increasing wildfire severity. Drought-induced woody plant dieback is a key driver of plant community shifts in woody encroached areas which may exacerbate fire hazard by increasing the ratio of dead to live plant material in shrublands. For example, in a *Juniperus-Quercus* woodland in Texas, United States, 22% of total woody plants died after drought events, with a noteworthy mortality of 30% of the evergreen shrub *Quercus pungens* (Twidwell et al. 2014). Also, an overall shrub mortality of 10.4% in *Pinus ponderosa* – dominated and pinyon juniper ecotone in northern Arizona, United States, was observed after the severe 2002 drought (Koepke et al. 2010). In our study, the drought year of 2011/2012 seems to have induced high *C. ladanifer* mortality, in spite of this species being considered drought-tolerant (Caldeira et al. 2015). However, a very dry year that may have been exacerbated by a high intra-specific competition for water (Roques et al. 2001, Galiano et al. 2010) due to the high shrub density at the site (e.g. 26000 individuals per hectare in ungulate-excluded plots) probably explains the high *C. ladanifer* mortality. Conversely, after the drought event, reduced competition for water resources due to *C. ladanifer* adult mortality may have also promoted high seedling recruitment (Del Cacho and Lloret 2012), explaining the high recruitment of *C. ladanifer* in our plots in 2013. This high recruitment also suggests a self-replacement ability of the dominant species C. ladanifer in our ungulate-excluded plots, rather than a consistent species shift (Martínez-Vilalta and Lloret 2016). Nevertheless, by the end of the experiment, in 2015, more than 60% of the emerged *C. ladanifer* young individuals had died, probably due to density-dependent mortality (Lambers et al. 2002), in the ungulate-excluded plots. The lack of *C. ladanifer* young individuals in the shrub management treatment plots (i.e. ungulate-exposed plots) may have resulted from a depleted seed bank caused by deer browsing (Lecomte et al. 2016) and the ongoing ungulate browsing pressure. Ecosystem resilience to effects of drought and browsing depends on timing of drought, the recovery capacity of the vegetation and the browsing pressure observed in the area (Ruppert et al. 2015). Drought, together with browsing, may even lead to vegetation collapse, as it has been shown after long-term chronic over-browsing (Goetsch et al. 2011)

Additionally, by increasing mortality and decreasing the live to dead ratio of plant material in *C. ladanifer* stands, drought strongly increased fire hazard. Moreover, changes in fuel characteristics in encroached *C. ladanifer* stands subject to drought led to an increased probability of transition of surface to tree crown fires which would have a critical effect on adult oak mortality (Lecomte et al. 2017 in prep). To our knowledge, no previous study addressed the effects of woody understory dieback on wildfire hazard as we did here.

Our study also emphasized that ungulate browsing acted together with drought to affect *C. ladanifer* mortality. Browsing increased *C. ladanifer* mortality and decreased shrub live-to-dead ratios. Grazing and browsing by sheep, together with drought, increased shrub mortality in Western Australia (Watson et al. 1997). Such changes in live to dead plant material caused by drought will significantly change shrubland fire hazard and need to be considered when considering ungulate ecology and fire hazard. Within the context of climate changes, under which higher frequency of extreme events such as droughts and fire are expected to occur, it is increasingly important to highlight how ungulate ecology, and particularly ungulate browsing, may jointly act with such events to affect ecosystems and the services they generate. Increased research on synergic effects of drought, fire hazard and ungulate browsing as drivers of ecosystem dynamics are increasingly needed.

The study revealed that shrub encroachment and drought strongly interacted to increase fuel load and wildfire hazard which may have important implications for ecosystems functioning. Management strategies, as ungulate browsing, to mitigate efficiently fire hazard need to be consider.

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CHAPTER 6

SYNTHESIS AND GENERAL DISCUSSION

5.6 Synthesis and general discussion

Shrub encroachment into grasslands and savanna-type of ecosystems has implications for their sustainable management. Replacement of grasslands by shrublands, for example, may constrain ecosystem functioning and the delivery of ecosystem services such as livestock production, which is supported by grasslands around the world, and affect wildlife species dependent on grassland habitat types (Bugalho and Milne 2003).

Shrub and woody plant species can also be consumed by domesticated and wild herbivores. Recently, Estell et al. (2012) reinforced the need to enhance shrub use by ruminants to meet global livestock production demands. However, large herbivores use of woody and other plant species, can induce extensive ecosystem changes, namely on their structure and species composition, affecting properties as animal trophic relationships, plant population dynamics, or the susceptibility of ecosystems to fire hazard or to store carbon (Stewart 2001, Hobbs 2006, Côté et al. 2014, Davis et al. 2016).

In relation to trophic interactions, use of plant resources by herbivores has strong implications on the magnitude and direction of such relationships. Herbivory can imply direct costs to plants (e.g. leaf and stem consumption, flower and bud predation) (Huffman and Moore 2003) but also benefits (e.g. plant overcompensation, seed dispersal) (Paige 1999, Levey et al. 2001). Moreover, benefits and costs to plants, will be shaped not only directly by herbivory but also through indirect effects of herbivory on multi-species interactions including insect, rodent or bird species (Fedriani et al. 2004, Rodríguez-Rodríguez et al. 2017). The overall direction and magnitude of multi-species interactions on plant populations is also dependent on the environmental context (e.g. plant species, population densities of herbivores, climate events such as droughts). These interactions need to be considered and integrated on a long-term basis, such as done in this thesis, to understand the dynamics of shrub encroachment (Chapter 2).

By altering the structure of woody plant communities (Chapter 3), herbivory, also affects ecosystems services such as carbon storage or fire hazard. Strong biomass reduction induced by long-term ungulate herbivory, such as shown in this thesis, will dramatically reduce above ground carbon storage but also substantially decrease the risk of severe wildfire. Moreover, by reducing the probability of crown fires and thus adult tree mortality, ungulate herbivory, may be indirectly favoring long-term carbon storage (Chapter 4). All these effects are constrained by climate conditions, such as drought events, which may act together with herbivory to increase woody plant mortality, change fuel characteristics, and affect fire hazard (Chapter 5).

Understanding the ecological mechanisms affecting shrub population dynamics and shrub encroachment, as well as the consequences for ecosystem functioning and ecosystem services is therefore essential for the sustainable management of encroached ecosystems.

This thesis advances the knowledge on how multiple plant-animal interactions (i.e. wild ungulates, insect seed predators and mice) affects the reproduction and population dynamics of *Cistus ladanifer*, a common native shrub to the western Mediterranean Basin, and thus its potential to encroach evergreen oak woodlands. The thesis also provide data on how wild ungulates mediate trade-offs between carbon stocks and wildfire hazard (a growing concern in Mediterranean fire prone ecosystems), also addressing how drought events, which will increase in frequency within the context of climate change scenarios, may act together with ungulate browsing to affect fire hazard.

The thesis, based on data (2001-1015) from an ongoing long-term study, clearly shown the ability of *C. ladanifer* to encroach in non-managed areas where ungulate browsing has been excluded. (fig. 1).

Figure 1. Shrub encroachment by *Cistus ladanifer* in the context of land abandonment (i.e. ungulate exclusion). No shrubs were present in 2001, year of the ungulate-exclude plots set-up. In summer 2003,

small shrubs were observed in the plots (A). Encroachment occurred through the years, leading to a high shrub density in 2016 (B).

Ungulate browsing and the reproduction behavior of C.ladanifer

Vertebrate and invertebrate seed predation, which often occurs during the pre-dispersal phase, can drastically limit recruitment and dynamics of plant populations (Mezquida and Benkman 2010, van Klinken and White 2014). In the case of woody and shrub plant species this may be a strong determinant of shrub encroachment which needs to be understood. Effects on seed predation are particularly important when multiple predators act synergically over time (Morris et al. 2007). *C. ladanifer* can be predated by large (e.g. deer, sheep) (Bugalho and Milne 2003, Mendes et al. 2015) or small mammals (e.g. mice) and invertebrates (e.g. beetles, ants) (Serrano et al. 2007, Teixido et al. 2011). This thesis has shown how several pre-dispersal seed predators can interact to influence the reproductive success of *C. ladanifer* (Chapter 2) and therefore its population dynamics and dynamics of encroachment. Data on seed predation and plant reproductive success (i.e. seed loss, fruit abortion) throughout the year and along plant phenology allowed quantification of seed losses due to ungulate and invertebrate herbivory. Ungulates, invertebrates and, to a lesser extent, seed-eating rodents had a cumulative effect on the reproductive success of *C. ladanifer*. Ungulates predated mainly reproductive structures and reduced seed production by nearly 42%. Additionally, 86% of the consumption occurred early in the season and incised on buds, flowers and immature fruits not allowing fruits to be developed (a plant cost). However 14% of the consumption occurred during summer and target mature fruits, that could eventually be dispersed by deer (a plant benefit) (Malo and Suárez 1995). In the absence of ungulates, insect pre-dispersal seed predators and rodents (only observed in the ungulate-excluded plots) reduced seed production by 40% and 6%, respectively. Additionally, 36% of the fruits aborted. This thesis especially emphasized the significant positive effect of ungulates on pre-dispersal insect seed predation that increased by 74.7%, probably due to a reduction in fruit availability to insects induced by deer browsing (Tarayre et al. 2007). Ungulates also increased seed loss in fruits depredated by insects by 32%, probably because fruits in ungulate-exposed plots presented less and lighter seeds that may have been consumed in a higher proportion by insects in order to fulfill their needs (Bonal et al. 2007). Finally, ungulates led to increased fruit abortion, by 60.9%, probably due to reduced pollinator visits (i.e. pollen limitation) resulting from lower flower availability (Sakata and Yamasaki

2015). These results suggest that multiple seed predators conferred strong costs to the reproductive success of *C. ladanifer*, which may have ultimately affected shrub population dynamics, particularly in the ungulate browsed areas.

Ungulate browsing and the population dynamics of C. ladanifer

The thesis also addressed the long-term outcome of the effect of high density ungulates on the population dynamics of *C. ladanifer* (Chapter 3) and data was collected on shrub volume, reproductive structures and seed production of *C. ladanifer* shrubs exposed or protected from ungulates during three reproductive seasons (i.e. 2007, 2008 and 2013). Specifically the thesis evaluated the effects of ungulates on *C. ladanifer* seed germination capacity, seed bank and population density. Results showed that ungulates strongly reduced shrub volume by 97% affecting flower bud production which, on average, reduced 96%. These patterns were consistent over the study years. Therefore, in the first year of this experiment (i.e. 2007) ungulates consumed more than 85% of C*. ladanifer* reproductive structures before July. These results reinforced previous findings showing that the benefits of seed dispersal were overridden by the early season ungulate predation of *C. ladanifer* flower buds and immature fruits. Additionally, seed mass and total seed number were higher in plants and fruits protected from ungulate browsing. These consistent reproductive costs, inflicted to *C. ladanifer* in the ungulate-exposed plots led to a distinct reduction of the soil seed bank which was, after 9 years, 2.6-fold higher in the ungulate-excluded plots than in the ungulate-exposed plots. This effect on seed bank was consolidated by our seed germination test that did not evidence differences in germination between treatments, discarding possible effects on seed viability (Chen et al. 2017).

As for many other Mediterranean plant species, growing under a high variable climate, *C. ladanifer* recruitment is highly dependent on the soil seed bank (Baskin and Baskin 2014). *C. ladanifer* seeds can persist viable in the soil over 7 years (Ferrandis et al. 1999, Clemente et al. 2007). Seed bank depression can affect plant population dynamics and resilience (Chaideftou et al. 2009, Pol et al. 2014). Ungulates had a clear and dramatic negative effect on *C. ladanifer* density after 12 years of browsing. Whereas no shrubs were present in 2001 and shrub density did not significantly differ in 2007 between treatments, shrub density decreased by 65% in the ungulate-exposed plot between 2007 and 2013 while it increased by 160% in the ungulate-excluded plot during the same period.

These first two chapters of the thesis clearly showed how multiple seed predators interacted to affect *C. ladanifer* population dynamics. Nevertheless, these results also reflected the high context-dependency of shrub-ungulate interactions. Although *C. ladanifer* is often considered as unpalatable species (Miranda et al. 2011), the high ungulate densities at the study site may have driven food competition and increased browsing pressure on *C. ladanifer* along the year. Increased browsed pressure in high population densities of ungulates has been observed in other studies (e.g. Arcese et al. 2014). Moreover, high ungulate population densities, such as is occurring in many regions of the Northern hemisphere particularly with deer, can modify ecosystem structure and affect the diversity of trees and other plant communities in forest ecosystems (Bugalho et al. 2013, Perea et al. 2014, Waller 2014). Such browsing effects may ultimately lead to cascading effects on other species including invertebrates (Bugalho et al 2011, Brousseau et al. 2012). Ungulate population density, through effects on habitat, may also affect vertebrate populations such as rodents (Keesing and Young 2014). This may contribute to explain, why rodent browsing was only observed in the ungulate-excluded plots where dense and well developed *C ladanifer* may have provided habitat cover. Moreover, *C. ladanifer* is known to constrain herbs and grasses productivity (Chaves et al. 2001, Herranz et al. 2005). Therefore an high ungulate browsing pressure, limiting *C. ladanifer* expansion and biomass production, may indirectly favor grass abundance and diversity (Diaz-Vila et al. 2003).

Ungulate browsing, shrub encroachment and ecosystem services

Shrublands generate a range of ecosystem services, including carbon storage and livestock forage (Papanastasis et al. 2008, Azorit et al. 2012). However, biomass accumulation, resulting from shrub encroachment and increased carbon stocks (Hughes et al. 2006, Alías et al. 2015) may trade-off with higher wildfire hazard, particularly in Mediterranean areas (Fernandes 2009). The thesis also aimed to understand how long-term ungulate browsing may mediate C storage and wildfire mitigation ecosystem service trade-offs (Chapter 4). Data was collected to quantify understory carbon accumulation (i.e. shrubs, herbs and litter) in 2007 and 2015 in ungulate-excluded and ungulate allowed plots. Shrub fuel was also characterized and fire behavior, as well as effects of fire on oak mortality, modeled in browsing and browsing excluded treatments. After 14 years of continuous ungulate browsing, ungulates had reduced horizontal (i.e. living shrub density by nearly 90%) and vertical (i.e. maximum height by 86%) structure of *C. ladanifer* population. Ungulates also decreased shrub biomass by 97% which, together with vegetation structure, is a wildfire driver (Fernandes 2001, De Luis et al. 2004). Additionally, by reducing shrub population structure and biomass (Sankaran et al. 2013, Veblen et al. 2015) but also litter (by 50%), ungulates reduced total understory carbon stocks by 90%, affecting an important ecosystem service. Nevertheless, these modifications of shrub structure and biomass led to a 80% reduction of the fine fuel which is a major driver of fire spread and ignition (Rothermel 1983). Consequently, ungulate browsing not only reduced potential fire spread rate and intensity (by 50% and 90% respectively) but also flame length and the likelihood of surface-to-crown fire transition. Such changes also implied a lower likelihood of adult oak mortality, which are the main ecosystem C storage reservoirs, and therefore a positive effect on the long term ecosystem carbon storage induced by ungulates. These results emphasize the potential of using wild ungulates to mitigate fire hazard as already suggested by others (Davies et al. 2010, Kimuyu et al. 2014, Lovreglio et al. 2014). Trade-offs between C stock reduction and fire mitigation showed that ungulates may not only prevent drastic C losses resulting from wildfire but also that C stock retained after wildfire may remain higher in browsed areas. Long-term benefits of ungulate browsing on carbon storage need to be equated with potential negative effects of browsing on tree regeneration (Bugalho et al. 2013) or damages to adult trees (Ramos et al. 2006). The thesis results suggest that further research on the balance between impact of ungulates on wildfire hazard and the carbon balance are clearly needed.

Ungulate browsing, shrub encroachment and drought

Climate change scenarios predict an increase in frequency and severity of extreme droughts, particularly in Mediterranean regions (IPCC 2014). Drought may induce high plant mortality including that of woody plant species (Allen et al. 2010). Higher plant mortality in turn will increase fuel availability and plant flammability through reduction of plant moisture, increasing wildfire hazard and severity (Anderegg et al. 2013, Littell et al. 2016). Thesis included dada collected during the year of 2011-12 which was an abnormally dry year (Trigo et al. 2013, Caldeira et al. 2015) allowing the study of the combined effects of drought and ungulate browsing on *C. ladanifer* populations and fire hazard (Chapter 5). Indeed, drought induced shrub mortality in 2012 and dead but standing *C. ladanifer* individuals could yet be observed in early 2013 and 2015, when shrub mortality reached 60% in the ungulate-excluded plots. Jointly effects of ungulates and drought increased *C. ladanifer* mortality by over 670% in 2013 and over 40% in 2015 (Fig. 2). Drought induced plant mortality and a drastic decrease of shrub density. Indeed in the ungulate-excluded plots, drought reduced living population density by nearly 10% and 60% in 2013 and 2015 respectively. Drought and ungulates had a cumulative effect on *C. ladanifer* populations reducing living shrub density by 70% and 84% in 2013 and 2015 respectively. Interestingly, drought also seemed to have positively affected *C. ladanifer* seedling recruitment a process that deserves further research in the future (Del Cacho and Lloret 2012). Data shown that in 2013, *C. ladanifer* seedlings accounted for 38% of the total living shrub population in the ungulate-excluded plots. Additionally, in 2015, seedlings accounted for 27% of living individuals but 67% of mortality was already observed within the seedling population, possibly due to density-dependent mortality (Lambers et al. 2002). Although, it is not the case of present study, research has shown that ungulate browsing, jointly with drought, may increase plant mortality in such a way to lead to vegetation collapse (He et al. 2017). In the study area, in the ungulate-exposed plots, seedling recruitment was very limited (8% of the total living population, on average) possibly due to the reduced seed bank (Chapter 3) and the ongoing browsing pressure. Finally, the thesis also showed how drought, through induced plant mortality, may increase fire hazard. The ratio of dead to live fine fuel increased over 600% in the ungulate-excluded plots and by 4000% in the ungulate-exposed plots, after drought, which increased fine fuel by 58% and 32% in the ungulate-excluded and ungulate-exposed plots respectively. This shift of fuel load structure increased of all wildfire severity parameters (i.e. surface rate of spread, fireline intensity and flame length) in both browsing treatments. However, ungulate browsing dampened the drought negative effects on flame length and intensity of wildfire, which did not increase as much as observed in the ungulate-excluded plots. Increase fire severity after drought may ultimately negatively affect the tree population (e.g. mortality). These results enhance the importance of ungulate ecology within the context of climatic changes.

Conclusions

The thesis revealed how multiple predators, especially wild ungulates, affect the reproduction and population dynamics of *C. ladanifer*, highlighting that shrub encroachment processes may underly on a complexity of ecological interactions.

Additionally, the thesis also highlighted that ecosystem services generated by shrub encroachment are mediated by ungulates which, although usually associated with negative effects on tree regeneration and survival may, through mitigation of fire hazard and crown fires, paradoxically contribute to tree survival. Finally, all these processes will be exacerbated within the context of climate change. Higher frequency and intensity of droughts will act together with ungulate browsing to affect shrub encroachment and risks of severe wildfires. Such relationships are particularly relevant in Mediterranean regions, such at that of the study area, which are an hotspot for climatic changes and where severity of wildfires has increased in recent decades.

Future research on shrub encroachment and management of encroached areas, especially in the fire-prone Mediterranean regions, should encompass ungulate ecology and its effects on the dynamics of shrublands and services these ecosystems may generate.

Figure 2. A) Ungulate-exposed plot in late summer 2011 and B) same ungulate-exposed plot in early spring 2013. Drought hydrological year 2011-2012 had a dramatic effect on *Cistus ladanifer* mortality and browsing by ungulates, which may have been exacerbated by the limiting feeding resources of herbs and grasses during the drought year. (Note the paired ungulate-excluded plot at the back).

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