DISTRIBUTION AND NATURAL ESTABLISHMENT OF *EUCALYPTUS GLOBULUS* IN THE IBERIAN PENINSULA: INSIGHTS INTO PROCESSES AFFECTING PLANT ESTABLISHMENT

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THESIS PRESENTED TO OBTAIN THE DOCTOR DEGREE IN
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Jury

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Abstract

Eucalypt plantations expanded across many countries and became subject to controversy, particularly about their ecological impacts and invasive potential. The same is true in the Iberian Peninsula (Iberia) regarding *Eucalyptus globulus* Labill. This thesis is composed by six studies (chapters) tackling poorly explored aspects in these domains, with the following objectives: a) to evaluate potential future dynamics of *E. globulus* plantations in Iberia according to different climate change scenarios and possible conflicts with high biodiversity areas (Chapter 1); b) to explore the influence of different factors on the natural establishment of *E. globulus* (Chapters 2-5); c) to perform a review of the literature investigating the natural establishment of eucalypt species (Chapter 6).

In Iberia, under both climatic scenarios, the suitable range of *E. globulus* plantations is expected to shrink and conflicts with high biodiversity areas may aggravate (Chapter 1). A countrywide survey in Portugal to estimate *E. globulus* recruitment, using Google Street View, showed that recruitment is mostly influenced by climatic variables (annual precipitation and thermal amplitude) and that Google Street View is a cost-efficient alternative to car surveys (Chapter 2). Field surveys in *E. globulus* plantations in Central Portugal showed abundant recruitment along plantation edges, influenced by local factors such as soil cover and tree age. Wildlings, mostly adult, are spread up to 76 meters from plantations (Chapter 3). A sowing experiment using *E. globulus* seeds showed that germination and survival was enhanced after harrowing (Chapter 4). A seed predation experiment revealed that *E. globulus* seeds are highly attractive but they have escaped in many locations (Chapter 5). The literature review retrieved 37 studies, addressing 61 eucalypt species in seven countries. Key factors influencing eucalypt recruitment include fire, propagule pressure and disturbances (Chapter 6).

Keywords: Eucalypt plantations, exotic plants, climate change, naturalization, invasion

Resumo

As plantações de eucalipto expandiram em muitos países e tornaram-se objeto de controvérsia relativamente a impactos ecológicos e potencial invasor. O mesmo acontece na Península Ibérica (Ibéria) relativamente a *Eucalyptus globulus* Labill. Esta tese é composta por seis estudos (capítulos) abordando tópicos pouco explorados nestes domínios, com os seguintes objetivos: a) avaliar as potenciais futuras dinâmicas das plantações de *E. globulus* na Ibéria de acordo com diferentes cenários de alterações climáticas e possíveis conflitos com áreas de elevada biodiversidade (Capítulo 1); b) explorar a influência de diferentes fatores na regeneração natural de *E. globulus* (Capítulos 2-5); c) realizar uma revisão da literatura que investiga a regeneração natural das espécies de eucalipto (Capítulo 6).

Na Ibéria, em ambos cenários climáticos, a extensão adequada para *E. globulus* deve contrair enquanto conflitos com áreas de elevada biodiversidade podem agravar (Capítulo 1). Observações ao longo de Portugal para estimar a regeneração de *E. globulus*, usando Google Street View, mostraram que a regeneração é principalmente influenciada por variáveis climáticas (precipitação anual e amplitude térmica) e que o Google Street View é uma alternativa eficaz a observações de carro (Capítulo 2). Observações em plantações de *E. globulus* em Portugal Central revelaram abundante regeneração ao longo das bordaduras, influenciada por fatores locais como coberto de solo e idade das árvores. Plântulas, maioritariamente adultas, estendem-se até 76 metros das plantações (Capítulo 3). Uma experiência com sementeira de *E. globulus* mostrou que a germinação e sobrevivência foi favorecida depois de gradagem (Capítulo 4). Uma experiência de predação de sementes revelou que as sementes de *E. globulus* são muito atrativas, embora tenham escapado em muitos locais (Capítulo 5). A revisão da literatura reuniu 37 estudos, abordando 61 espécies de eucalipto em sete países. Fatores chave para o recrutamento de eucaliptos incluem o fogo, pressão de propágulos e perturbações (Capítulo 6).

Palavras-chave: plantações de eucalipto, plantas exóticas, alterações climáticas, naturalização, invasão

Resumo alargado

A partir de meados do século XX, as plantações de eucalipto proliferaram por vários países, cobrindo atualmente mais de 20 milhões de hectares. A par com este crescimento, os eucaliptos têm sido alvo de grande controvérsia e preocupação, em especial pelos seus impactos ambientais e potencial invasor nas áreas de introdução. O mesmo sucedeu na Península Ibérica (Ibéria), em particular com plantações de *Eucalyptus globulus* Labill., que quase quadruplicaram de extensão nas últimas décadas, passando de c. 340 mil hectares para 1,4 milhões de hectares entre 1970 e 2010. A crescente preocupação ambiental com os eucaliptos deu origem a vários estudos, em diferentes países, nos domínios dos impactos ambientais e potencial invasor, especialmente nos últimos anos. Na Ibéria, a investigação tem sido focada em *E. globulus*, ainda que esta espécie tenha sido investigada em outros países como Espanha, África do Sul, Chile, EUA, Brasil e Austrália. Apesar do crescente corpo de literatura nestes tópicos, nunca se tentou uma revisão da literatura, e há ainda questões importantes que têm sido pouco exploradas, algumas das quais são abordadas nesta tese.

Esta tese compreende um conjunto de seis estudos (Secção II) que pretendem dar resposta a questões relacionadas com as plantações de *E. globulus* e com fatores que poderão ajudar a compreender a regeneração natural da espécie nas áreas de introdução. Cinco destes estudos foram publicados em cinco diferentes revistas internacionais indexadas e, portanto, são apresentados sob a forma de artigo científico. Um sexto estudo foi desenvolvido para esta tese e pretende fazer uma revisão da literatura sobre a regeneração natural dos eucaliptos (todas as espécies) nas áreas de introdução, prevendo-se que futuramente origine um novo artigo.

O primeiro estudo (Capítulo 1) pretendeu estimar, na Ibéria, a atual e futura potencial distribuição de plantações de *E. globulus* de acordo com diferentes cenários de alterações climáticas, bem como dos possíveis conflitos com áreas de elevada biodiversidade. Usámos modelos de distribuição de espécies para projetar as distribuições das plantações, sobre uma grelha de 1x1 km sobre a Ibéria, com níveis de aptidão climática para as plantações em cada célula, de acordo com dois cenários de alterações climáticas: um mais pessimista (maior concentração de gases com efeito de estufa) e um mais otimista. Para classificar o nível de conflito entre plantações e áreas de elevada biodiversidade, combinámos, em cada célula, o nível de aptidão para plantações com o nível de importância

sítios da Rede Natura 2000 ou áreas agrícolas de elevado valor natural (‘high nature value farmlands’). Os modelos mostram que a temperatura mínima do mês mais frio foi a variável mais importante para a distribuição das plantações. Em ambos os cenários climáticos, a extensão apta para plantações contrai-se, com uma ‘migração’ das plantações para norte, muito mais expressiva no pior cenário, embora a aptidão climática melhore na generalidade, o que pode fomentar o estabelecimento de plantações e o agravamento dos conflitos com áreas de elevada biodiversidade. Este estudo foi publicado na revista *Journal for Nature Conservation*.

O segundo estudo (Capítulo 2) teve um duplo objetivo: a) identificar as variáveis (regionais e locais) que influenciam a abundância de regeneração natural de *E. globulus* em Portugal continental; b) avaliar a eficácia do Google Street View (GSV) como método de amostragem, comparando os modelos produzidos (usando Árvores de Regressão Impulsionada - *Boosted Regression Trees*) da abundância da regeneração natural através de levantamentos de carro e por GSV. Neste estudo replicámos, usando GSV, observações de carro para estimar a abundância de regeneração natural de *E. globulus* nas bermas de estrada adjacentes a plantações. Adicionalmente, através do GSV, conseguimos recolher novas variáveis locais, como o tipo de povoamento (puro; misto), os cobertos de solo e declive. Ambos os métodos produziram modelos semelhantes, identificando as mesmas variáveis explicativas e com semelhante influência na regeneração natural. As variáveis locais aumentaram a performance do modelo e melhoraram a nossa perceção sobre os fatores que influenciam a regeneração natural. O GSV revelou-se uma alterativa eficiente aos levantamentos de carro. Este estudo foi publicado na revista *Environmental Monitoring and Assessment*.

O terceiro estudo (Capítulo 3) pretendeu investigar a influência de fatores locais na presença e abundância da regeneração natural de *E. globulus* nas bordaduras das plantações, bem como avaliar o tamanho e estado reprodutivo das novas plântulas. Adicionalmente, investigámos as distâncias de dispersão e o seu estado de desenvolvimento das novas plantas de eucalipto. Para tal, em dezenas de plantações em Portugal Central, realizámos levantamentos ao longo das bordaduras e nos habitats adjacentes. Metade dos 1.630 eucaliptos resultantes de regeneração natural (recrutas) presentes nas bordaduras tinham mais de 1.3 metros de altura e c. 8% atingiram a maturidade sexual (tinham flores ou cápsulas). A proporção de recrutas reprodutivos foi positivamente influenciada pelo tamanho das plantas, pela localização fora das plantações e por menor número de dias de geada. Fora das plantações, os recrutas foram encontrados até ao máximo de 76 metros de distância. Concluímos que as distâncias de dispersão foram influenciadas positivamente pela direção dominante dos ventos e pela presença de águas de escorrência. Este estudo foi publicado na revista *Biological Invasions*.

O quarto estudo (Capítulo 4) pretendeu compreender o efeito de ações de gestão normalmente realizadas dentro e fora das plantações na regeneração natural de *E. globulus*. Realizámos uma sementeira com sementes 'livres' e sementes 'encapsuladas' (dentro de cápsulas de eucalipto) de *E. globulus*, em áreas de 0,5 por 0,5 metros, sujeitas a 16 tratamentos diferentes, resultado de um desenho fatorial completo entre três fatores: operações de gestão (gradagem; nivelamento do solo com lâmina; fogo controlado; e parcelas de 'controlo'); tempo de sementeira (antes ou depois dos tratamentos); tipo de propágulos (sementes ou cápsulas com sementes). Cada tratamento foi replicado 10 vezes e as parcelas foram monitorizadas durante um ano. Registámos 4,8% de germinações das sementes (379 em 8.000 sementes), mas as 800 cápsulas originaram apenas duas plântulas. Um mês e meio depois da sementeira, 96,3% das plantas haviam germinado. As análises dos dados (Árvore de Regressão Multivariada e Modelos Lineares Generalizados Mistos) mostraram que a germinação foi mais elevada nas parcelas semeadas após lavragem do solo, e que parcelas semeadas antes do fogo tiveram o efeito oposto. As probabilidades de sobrevivência (Modelos de Cox) das plântulas foram mais elevadas nas sementeiras após tratamentos, e especialmente após a lavragem. A mortalidade das plântulas ao longo do tempo (Modelos Lineares Mistos) foi negativamente influenciada pela lavragem do solo e positivamente pelos valores de evapotranspiração da cultura. Este estudo foi publicado em setembro de 2019 na revista *Forest Ecology and Management*.

O quinto estudo (Capítulo 5) pretendeu investigar se a fauna local usa as sementes de *E. globulus* e a possível influência na regeneração natural desta espécie. Para tal, preparámos uma experiência de predação usando sementes de *E. globulus* e, para efeitos comparativos, duas outras espécies comuns no área de estudo - *Acacia dealbata* Link e *Cistus salviifolius* L. Distribuímos comedouros por três habitats diferentes junto à Escola Superior Agrária de Coimbra (eucaliptal, carvalhal e acacial), e aplicámos diferentes tratamentos de exclusão nos comedouros (exclusão de vertebrados; exclusão de invertebrados; sem exclusão; exclusão total – 'controlo), para identificar o tipo de animais e 'controlar' agentes externos como o vento. As sementes de *E. globulus* revelaram-se bastante atrativas para vertebrados e invertebrados, tendo sido posteriormente identificadas diferentes espécies de formigas e uma espécie de rato. Através de Análise de Componentes Principais, descobrimos forte disparidades espaciais no tipo de predadores e intensidade de predação, o que pode ajudar a compreender a heterogeneidade espacial na regeneração natural de *E. globulus* observada noutros estudos. Este estudo foi publicado na revista *Web Ecology*.

O sexto estudo (Capítulo 6) pretendeu realizar uma revisão da literatura sobre a regeneração natural dos eucaliptos nas áreas de introdução, tentando proporcionar uma visão geral da literatura, identificar os principais fatores que influenciam a regeneração natural dos eucaliptos e tentar sintetizar o conhecimento atual. Esta revisão da literatura teve por base uma extensa pesquisa bibliográfica para reunir a analisar os estudos que realizaram observações no terreno para estudar a regeneração natural dos eucaliptos por todo o mundo, incluindo todas as espécies dos géneros *Eucalyptus*, *Corymbia* e *Angophora*, Recolhemos um total de 37 estudos, investigando 61 espécies de eucalipto em sete países (Portugal, África do Sul, Brasil, EUA, Espanha, Austrália e Chile). As espécies mais frequentemente abordadas foram, por ordem de importância, *E. globulus*, *E. grandis*, *E. camaldulensis*, *E. cladocalyx*, *E. tereticornis*, *E. robusta* e *E. saligna*. A regeneração natural reduz drasticamente desde o interior das plantações até zonas distanciadas 50-100 m das plantações, embora alguns estudos tenham registado plantas até 75-76 metros de distância das plantações. O clima é um fator importante, mas a sua resolução pode ser demasiado grande para identificar com precisão os locais mais favoráveis onde ocorre a regeneração natural, pois as variáveis locais têm muita importância, como a pressão de propágulos (associada à idade das plantações), a competição com outras plantas (coberto de solo) e a ocorrência de perturbações como incêndios ou ações de gestão. Prevê-se que este estudo origine futuramente uma nova publicação.

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SECTION I: General Introduction

1 Foreword

The present thesis, titled ‘Distribution and natural establishment of *Eucalyptus globulus* in the Iberian Peninsula: insights into processes affecting plant establishment’, was prepared under the Doctoral Programme ‘SUSFOR - Sustainable Forests and Products’, held at the School of Agriculture, University of Lisbon (*Instituto Superior de Agronomia*, *Universidade de Lisboa*). This work was supported by a PhD. scholarship funded by the Portuguese Foundation for Technology and Science (*Fundação para a Ciência e Tecnologia*; PB/BD/113936/2015).

The backbone of this thesis is a set of six studies, five of which published in indexed (Web of Science) journals, resulting from the collaboration between several researchers from different institutions. Most studies presented here make part of an ongoing investigation which started with project WildGum (PTDC/AGR-FOR/2471/2012), aimed at investigating the naturalisation of *E. globulus* Labill. in Portugal, based on a multi-scale approach for a comprehensive understanding of the naturalisation process and its mechanisms. That project has now a successor, the project WildGum II (PDR2020-101-030919), which started in October 2018, aimed at investigating *E. globulus* invasion based on satellite imagery and genetic markers.

I collaborated in project WildGum as a scholarship fellow between October 2013 and April 2015, by performing mostly field work (data collection) in different studies. Some of these studies supported my successful application for a PhD. Two of those studies become the current Chapters 3 and 4 of this thesis. During my time in WildGum, I helped designing two new studies not originally planned in that project, which also integrate this thesis as Chapters 2 and 5.

The first chapter of this thesis does not make part of the WildGum legacy, but instead resulted from a new collaboration that emerged in COST Action FP1403: Non-native tree species for European forests - experiences, risks and opportunities (NNEXT), through my supervisor J. S. Silva. I accepted the kind invitation to lead the writing of the paper as the study was thought to be a valuable asset and complement to my work. I was also challenged by J. S. Silva to conduct a literature review on the natural establishment of *E. globulus* in the introduced range, which was later extended to all eucalypt species. The resulting work is to be submitted to an international journal, but for the moment it is written and formatted as standard book chapter and corresponds to Chapter 6 of this thesis.

Some of these works were presented in international conferences. The study in Chapter 1 was presented at the '2017 *World Conference on Natural Resource Modeling* (WCNRM 2017)' (Deus et al. 2017) and later at the '*International Conference: Non-native tree species for European Forests* (NNEXT)' (Deus et al. 2018b). The study in Chapter 3 was presented at the '*14th International Conference on Ecology and Management of Alien Plant Invasions* (EMAPI 2017)' (Deus & Silva 2017). Some of our works also got media attention and were published in some printed and online newspapers. It was the case of the studies from Chapters 1 and 3[1].

Apart from the works presented in this thesis, I co-authored a paper published in 2017 (Águas et al. 2017), aimed at investigating *E. globulus* recruitment along plantation edges (which I helped surveying) and comparing the results with a similar study conducted previously in Australia (Larcombe et al. 2013). I collaborated in another manuscript, led by M. Tomé, currently under review in the journal *Forestry*, performing a review on *E. globulus* plantations in Europe, including their management and ecology. Also, I am collaborating in another manuscript, led by L. Queirós, mostly aimed at modelling and projecting the risk of natural establishment of *E. globulus* throughout the Iberian Peninsula, relying strongly on the results from the studies presented in the Chapters 1 and 2 of this thesis.

2 Problem statement

Eucalypts are nowadays one of the most popular trees worldwide. Eucalypt plantations expanded in many countries since the mid-20th century, becoming a conspicuous element in the landscapes of many countries from tropical, subtropical and temperate zones (Rejmánek & Richardson 2011; Stanturf et al. 2013). Currently, eucalypt plantations should cover more than 20 million ha outside the native range (Rejmánek & Richardson 2011). However, eucalypts also became one of the exotic trees subject to more controversy and heated discussion between different stakeholders, particularly about their ecological impacts (see, for instance, Casson 1997; Dessie et al. 2019; Jagger & Pender 2003; Madalcho et al.

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[1] Examples of online articles about these studies can be found in the following links (accessed September 2019):https://24.sapo.pt/atualidade/artigos/alteracoes-climaticas-vao-tornar-cultivo-do-eucalipto-impraticavel-a-sul-do-tejo (Chapter 1); www.dn.pt/lusa/interior/estudo-apresenta-novas-evidencias-do-potencial-invasor-do-eucalipto-10692251.html (Chapter 3)

2019; van Wilgen 2012; Wolf & DiTomaso 2016). In one hand, there is a general perception that eucalypt plantations can cause noxious environmental impacts, an assumption supported by many studies (e.g. Amenu 2017; Banfield et al. 2018; Becerra et al. 2018; Dzikiti et al. 2016; Ferraz et al. 2019; Hoogar et al. 2019). On the other hand, it is frequently argued that eucalypt plantations can alleviate the pressure on native forests by allowing them to be kept for conservation purposes, and that, given an adequate, ecologically-oriented management, negative environmental impacts can be attenuated or even reverted (Amazonas et al. 2018; Brancalion et al. 2019; Brockerhoff et al. 2013; Emer & Fonseca 2011; Vance et al. 2014).

The Iberian Peninsula (Iberia) is home to similar controversy and contention about the potential ecological impacts of eucalypts[2], especially about *Eucalyptus globulus* Labill., which has been the subject of many studies (e.g. Abelho & Graça 1996; Barrocas et al. 1998; Calviño-Cancela et al. 2012; De la Hera et al. 2013; Rodríguez-Suárez et al. 2011; Souto et al. 2001). In Iberia, since the mid-20th century, following the increasing demand for *E. globulus* to feed the pulp industries, there was a massive, uncontrolled (even if regulated) proliferation of *E. globulus* plantations, mostly propelled by the individual choices of hundreds of thousands of private landowners, causing major landscape transformations (Fig. 1). However, climate change may lead to a compulsory rearrangement of the distribution of *E. globulus* plantations across Iberia, since the species (and plantations) may be forced to 'migrate', similarly to many species around the world (Harrison et al. 2006; Hickling et al. 2006), including tree species in the Iberian Peninsula (Garzón et al. 2008). In this regard, Iberia is expected to be one of the most responsive regions to climate change (Giorgi 2006). Given the socioeconomic importance of this species in many Iberian regions, it is of uppermost importance to anticipate how climate change may impact plantation productivity and the suitable range for cultivation. Likewise, the shift of the suitable range for *E. globulus* plantations may give rise to new environmental risks, like the replacement of valuable habitats, as seen before in Iberia (Abelho & Graça 1996; Acácio et al. 2016; Pozo et al. 1998), and the disruption of habitat connectivity (Fahrig 2003; Taylor et al. 1993;

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[2] In the late 2018, over one hundred Portuguese personalities from different backgrounds (academic, political, industry) subscribed a manifest defending that trees (including eucalypts) should not be discriminated. Some months later, a smaller group of personalities, mostly from academic background and NGO's, replied with a manifest defending that trees should be discriminated, particularly a positive discrimination of native trees.

Teixido et al. 2010), thus jeopardising conservation goals. It is thus important to anticipate such future dynamics to facilitate and encourage a healthy conciliation between economic and environmental interests.

One of the disputed aspects about eucalypt plantations is their invasive potential, which is frequently contested because eucalypts are, admittedly, less invasive than other problematic trees, such as *Acacia* spp., *Pinus* spp. and *Prosopis* spp (see, for instance, comparisons between eucalypts and pines: Calviño-Cancela & van Etten 2018; Fernandes et al. 2016). The reasons frequently pointed out, to explain why eucalypts are less successful invaders than species as the ones exemplified above, include the reduced dispersal capacity, the inability to form long-living seed banks, the high mortality rates of young eucalypts and the lack of compatible ectomycorrhizal fungi (Rejmánek & Richardson 2011).

However, there has been a growing body of empirical evidences of eucalypt invasion (e.g. Becerra 2006; Fork et al. 2015; Forsyth et al. 2004; Ruthrof et al. 2003) and some countries already devote many resources to control eucalypt invasion (Andreu et al. 2009; van Wilgen et al. 2012). Reviews of national and international databases identified several eucalypt species which became ‘naturalised’ (Pyšek et al. 2017) and a few species recognised as ‘invasive’ in different regions of the world (Haysom & Murphy 2003; Rejmánek & Richardson 2013). Additionally, weed risk assessments conducted in different countries flagged many eucalypt species as potential high risk invaders (Gordon et al. 2012; Jaryan et al. 2013; Nel et al. 2004). It is important to clarify the concepts of ‘naturalised’ and ‘invasive’ species. Richardson et al. (2000) defined ‘naturalised’ plant species as species able to generate sexually mature offspring, normally nearby parent plants, enabling the formation of self-perpetuating populations, while ‘invasive’ plant species were defined as naturalised species able to originate reproductive offspring at considerable distances, specified as a distance of 100 m in 50 years for plants spreading by seeds like eucalypts.

Awareness about the invasive behaviour of eucalypts is relatively recent. Perhaps the first reference to eucalypt invasion dates from the 1950’s, when addressing the impacts of *E. globulus* in California (Oosting 1956, cited by Richardson 2011b). Only twenty years later, in 1975, the first systematic surveys on eucalypt recruitment were conducted (Kirkpatrick 1977). In the 1980’s and 1990’s, surveys on invasive plant species (including eucalypts) were conducted across South Africa by L. Henderson (e.g. Henderson 1989; Henderson 1998; Henderson & Musil 1984). In the 2000’s, only a few studies performed field observations to assess the invasive potential of eucalypts (e.g. Ritter & Yost 2009; Ruthrof et al. 2003), but since 2011, these studies multiplied, targeting several eucalypt species in different countries, such as Brazil, USA and Australia (e.g. Callaham Jr et al. 2013; Larcombe et al. 2013; Miolaro et al. 2017). As a result, today we can find a large body of literature investigating eucalypt invasiveness and the factors influencing natural establishment in the introduced range. Yet, so far, no comprehensive review was attempted.

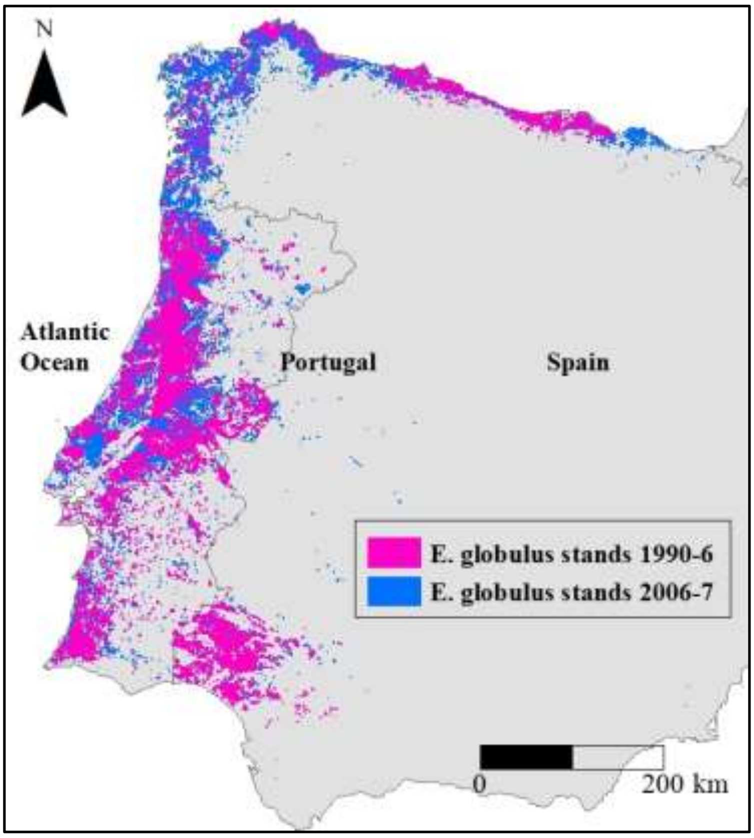

Fig. 1 - Distribution and dynamics of *Eucalyptus globulus* stands in the Iberian Peninsula between 1990-6 and 2006-7 (Portugal and Spain, respectively). Please note that, in order to increase the visibility of *E. globulus* stands at such small scale, the stands (shapefiles) were drawn (in GIS) with a narrow outline, meaning that their representation is magnified

The invasive potential of *E. globulus*, particularly in Iberia, has also been disputed. The species is not officially classified as 'invasive' in Portugal or Spain, even though it was included by researchers in national lists of invasive plants (Dana et al. 2004; Marchante et al. 2014). In the global review conducted by Rejmánek and Richardson (2013), *E. globulus* was the eucalypt species identified as invasive in the greater number of regions in the world. At the start of this PhD., only a few published had investigated the natural establishment of

E. globulus in Portugal (Águas et al. 2014; Catry et al. 2015) and Spain (Calviño-Cancela & Rubido-Bará 2013). Since then, other studies were published, which added valuable insights into the species' invasiveness and the factors influencing recruitment (e.g. Águas et al. 2017; Fernandes et al. 2017; Fernandes et al. 2018). Nevertheless, some key aspects remain poorly explored, such as: the development and onset of sexual maturity of naturally established eucalypts; the escape from plantations and the factors influencing the distance of dispersal; the effects of standard management operations on natural establishment; or the influence of seed-harvesting animals on the fate of dispersed seeds. Research on these topics would help understanding *E. globulus* recruitment and its invasive potential, in order to identify locations and situations of increased risk and help define monitoring and management plans.

3 Study species

The studies composing this thesis are mostly focused on *Eucalyptus globulus* Labill., which is the predominant eucalypt species in the Iberian Peninsula, southwest Europe, especially in Portugal. In the Chapter 6, we present a literature review targeting all eucalypt species which have been subject to field observations to assess natural establishment. A general description of eucalypts, including species from the genera *Eucalyptus*, *Corymbia* and *Angophora*, can thus be found in Chapter 6. In this section, we provide some relevant aspects regarding the species *E. globulus*, despite some traits being common to most eucalypts.

Eucalyptus globulus Labill. (Myrtaceae), commonly known as Tasmanian blue gum, is an evergreen tree, up to 60 m height, native to Tasmania, Bass Strait Islands and southern Victoria (Potts et al. 2004). *Eucalyptus globulus* was first discovered in Tasmania in 1792 by French explorers, and by the end of the 19th century it had been introduced to several countries (Potts et al. 2004). Nowadays, *E. globulus* is one of the most widespread eucalypt species (Rejmánek & Richardson 2011).

In its native region, *E. globulus* prospers in a relatively wide range of climatic and geographic situations. In fact, it is one of the eucalypt species with the largest native ranges (Hui et al. 2014). Kirkpatrick (1975) provides a detailed characterisation of the geographic, climatic, geological and edaphic ranges of the species. Across the native range, summers vary from mild to warm, and winters vary from cool to mild. The number of frost days vary from nil in coastal regions to over seventy days at the altitudinal limit of the species. As to precipitation, *E. globulus* is more frequent in regions registering between 600 to 1,000 mm of rainfall. However, it seems that local factors, such as aspect and topography, play a key role in the species distribution. For instance, *E. globulus* can be found in regions with less than 500 mm rainfall, but individuals are confined to south-facing slopes and along streams. It seems that *E. globulus* has no particular geological or edaphic requirements, as the species can be found on a wide diversity of geological substrates and soil conditions (Kirkpatrick 1975; Williams & Potts 1996).

Time to reach reproductive maturity in *E. globulus* ranges between 3-6 years (Potts & Gore 1995; Potts et al. 2008). Flowering levels are relatively low in plantations but higher within the individuals located at the edges of plantations (Barbour et al. 2008). In the native range, *E. globulus* flowers are mainly pollinated by insects and birds (Hingston & Potts 1998). The onset of sexual maturity is normally associated with the transition from juvenile to adult foliage (Potts & Gore 1995; Potts et al. 2008). *Eucalyptus globulus* leaves show a heteroblastic development. There are significant functional differences between juvenile and adult leaves (Velikova et al. 2008), but also differences in colour and shape (Johnson 1926). Juvenile leaves are normally, egg-shaped, sessile, arranged in pairs, horizontal and featuring a conspicuous glaucous colour. In contrast, adult leaves are alternate, lance-shaped, on a small petiole and dark green coloured. It is thus very easy to distinguish juvenile from adult leaves. Also, both juvenile and adult *E. globulus* individuals are easily distinguishable from the native flora in the Iberian Peninsula.

Seeds of *E. globulus* are relatively small (max. width c. 2 mm), non-dormant and short-lived, thus not forming a long-living soil seed bank (López et al. 2000; Santos et al. 2015). Seeds have no particular adaptation for long distance dispersal. Wind is the main agent of dispersal, but seeds can be dragged along drainage lines originating successful establishment at greater distances (distance of 175 m recorded by Larcombe et al. 2013). According to the calculations by Cremer (1977), *E. globulus* seeds (average weight of 3.03 mg) dropped from a height of 40 m, on a levelled surface, under a wind velocity of 10 km/h, would reach c. 20 m distance. In the same conditions, the light seeds of *E. deglupta* (average weight of 0.14 mg) would reach c. 53 m distance. In northwest Spain, Calviño-Cancela and Rubido-Bará (2013) were able to record field observations of seed dispersal, by establishing seed/capsule traps along a gradient of distance relatively to the limit of *E. globulus* plantations, featuring trees with an average height of 25 m. They estimated that 99% of *E.*

globulus seeds fall within 15 m from plantations, but the maximum distance recorded was 80 m. They hypothesise that capsules may be catapulted by the elastic branches of *E. globulus* trees in windy days, allowing to reach greater distances. Seeds can be released from capsules while on canopies or they can be released after capsules fall on the ground, sometimes forming little heaps of seeds (Bean & Russo 1986).

In the Iberian Peninsula, plantations are mostly cultivated as even-aged monocultures, normally coppiced every 10-12 years up to three rotations (Silva & Tomé 2016). *Eucalyptus globulus* resprouts from both the lignotuber and the stem(s) as a regeneration strategy after catastrophic events such as clearcutting or wildfires (Nicolle 2006). In Iberia, plantations are distributed across a climatic, geological and altitudinal gradient (Catry et al. 2015; Serrada et al. 2008), but greater densities of *E. globulus* plantations are normally found in the most productive regions (Tomé 2000).

4 Objectives

The objectives of the present thesis can be summarised in three major topics. The first objective was to estimate potential future dynamics of *E. globulus* plantations according to different climate change scenarios and possible conflicts with high biodiversity areas (Chapter 1). The second objective was to assess the influence of different factors on the natural establishment of *E. globulus* (Chapters 2-5). The third objective was to perform a review of the literature investigating the natural establishment of eucalypt species, with a particular focus on *E. globulus* (Chapter 6). Specific objectives are presented in each study.

5 Thesis structure

This thesis is composed by three Sections. In Section I (General Introduction), we try to provide a general introduction to the works presented in Section II (Studies), including a summary of these studies in subsection 6, where the reader may find, for each study, the main objectives, some methodological notes, some key results and publishing information.

Section II (Studies) gathers the set of studies produced in the frame of this PhD. thesis. The thesis includes a total of six studies, each corresponding to a single chapter. The first five studies (Chapters 1-5) were published in indexed (Web of Science), international journals. As a result, each of these studies is presented as an independent study following the standard structure of scientific papers. No changes were made to the contents of the original published texts. The final chapter follows a different structure as it corresponds to a literature review prepared specifically for this thesis, even though we expect to submit a refined version of that work to an international journal. Section III (General Discussion) is devoted to a general discussion of some of the issues tackled in this thesis, including some knowledge gaps and research needs, and to share some overarching insights about this work, its importance and possible contributions.

6 Summary of studies

This thesis includes six studies composing Section II (Studies). The first study (Chapter 1) explored the current and future conflicts between *E. globulus* plantations and high biodiversity areas in the Iberian Peninsula. We used species distribution models to project the current and future potential ranges of *E. globulus* plantations, featuring levels of environmental suitability, according to different climate change scenarios, corresponding to two ‘representative concentration pathways’ (of greenhouse gas): RCP2.6 (optimistic scenario) and RCP8.5 (pessimistic scenario). These projections were confronted with the distribution of Natura 2000 sites and ‘High Nature Value farmlands’ to rate potential conflicts based on the level of suitability for plantations and the importance of high biodiversity areas. Under both future scenarios, the range of *E. globulus* plantations shrinks, much more expressively under the worst scenario, but the suitability for plantations improves generally, leading to a potential aggravation of conflicts with high biodiversity areas. This study was published (online first) in the *Journal for Nature Conservation* in June 2018 (Deus et al. 2018a).

The second study (Chapter 2) tested the efficiency of Google Street View (GSV) as a survey method and investigated the influence of regional- and local-scale factors on *E. globulus* recruitment across Portugal. Using GSV, we performed a countrywide survey in Portugal to estimate the abundance of *E. globulus* offspring in roadsides adjacent to plantations. We revisited the areas surveyed by car in a previous study (Catry et al. 2015), but collected additional local-scale variables. Models of wildling abundance were produced for both survey methods using Boosted Regression Trees to compare model performance and the added value of local-scale variables. Models from both methods were similar in the selected variables, even though the GSV model had lower explained deviance. Mean annual precipitation and distance to the ocean (surrogate for thermal amplitude) were the most important variables. Local variables (e.g. slope; coppiced stand) were less important but improved model performance. This study was published (online first) in *Environmental Monitoring and Assessment* in September 2016 (Deus et al. 2016).

The third study (Chapter 3) aimed to investigate the influence of local-scale factors (together with regional-scale factors in some analyses) on the presence, abundance and development of *E. globulus* wildlings along the edges of plantations, and the factors influencing the distance of wildling establishment into adjacent habitats. We surveyed the edges and the adjacent habitats of several *E. globulus* plantations in Central Portugal. A total of 1,630 wildlings were recorded along the edges of plantations. Wildling densities were higher in the inner edges of plantations. Around 8% of the surveyed *E. globulus* wildlings possessed flowers or fruits, including some very small wildlings (<1.3 m). The proportion of reproductive wildlings was positively influenced by wildling size, the location in the outer edge of plantations, and negatively influenced by the number of frost days. Outside plantations, a total of 641 E. globulus wildlings were found up to a distance of 76 m from plantations, and the dispersal distanced was positively influenced by the direction of dominant winds and the presence of drainage lines. This study was published (online first) in *Biological Invasions* in February 2019 (Deus et al. 2019).

The fourth study (Chapter 4) explored the effects of standard vegetation management operations on the germination and early demography of *E. globulus* offspring. We performed a sowing experiment, using *E. globulus* seeds and capsules, subject to 16 different treatments (replicated 10 times), resulting from a full factorial design with three factors: ‘management operation’ (*soil harrowing*; *soil stripping*; *burning*; *control*); ‘sowing time’ (*before* or *after* the operation); ‘propagule type’ (*capsules* or *seeds*). Plots (0.5x0.5 m) were sowed with 100 seeds or 10 capsules and monitored along one year. Seed sowing resulted in 4.8% germination (379 out of 8,000 seeds) but only two seedlings originated from 800 capsules dropped. In 1.5 months, 96.3% of seedlings had emerged. Germination and survival was enhanced in seeds sowed *after harrowing*. Germination was lower in seeds sown *before burning*. Management operations performed after seed shed can significantly prevent germination. All seedlings died within a year. This study is currently in press in *Forest Ecology and Management* (Nereu et al. in press).

The fifth study (Chapter 5) explored the existence and possible influence of animal interaction with post-dispersed *E. globulus* seeds. We performed a seed predation experiment in Coimbra (Portugal) using seeds from *E. globulus* and, for comparison purposes, seeds of *Acacia dealbata* Link and *Cistus salviifolius* L. Seed predation was tested in three different habitats, each dominated by one of the studied species. In each habitat, we established 10 feeding stations, each composed by four feeders with different animal exclusion treatments (vertebrate, invertebrate, total and no exclusion). Each feeder was replenished daily with five seeds from each species. Feeders were monitored every 24 hours during nine summer days. *Eucalyptus globulus* seeds were highly attractive to both vertebrates and invertebrates (as much as *A. dealbata*, and more than *C. salviifolius*). There was high spatial variation regarding the predominant seed predators but many locations registered negligible seed predation. This study was published in *Web Ecology* in April 2018 (Deus et al. 2018c).

The sixth study (Chapter 6) performed a review of the literature assessing the natural establishment of eucalypt species and the factors influencing it outside the native range. A literature survey, using the Web of Science online database, gathered and analysed studies performing field surveys and experiments to investigate the natural establishment of eucalypt species from the genera *Eucalyptus*, *Angophora* or *Corymbia*. The literature survey retrieved 37 studies from seven countries, mostly conducted in the last years. *Eucalyptus globulus* was the most investigated species (20 studies in six countries) and received particular attention in this study. Other frequent species included *E. grandis*, *E. camaldulensis* and *E. cladocalyx*. Data retrieved from studies showed a decreasing average wildling density from the inside plantations (1,812 wildlings ha^{-1}) to areas distanced *50-100 m* from plantation limits (5.8 wildlings ha^{-1}). Recruitment curves show a sharp decrease of wildling densities outside plantations, but wildlings are spread up to 75-76 m from plantations. Key factors enhancing recruitment include higher propagule pressure, reduced plant competition and disturbances such as wildfires and management. This study was prepared specifically for this thesis, but a refined version is planned to be submitted later to an international journal.

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SECTION II: Studies

Chapter 1: Current and future conflicts between eucalypt plantations and high biodiversity areas in the Iberian Peninsula

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Author contribution:

a) helped conceiving the experimental design; b) collected and organised data in GIS on the distribution of *E. globulus* and high biodiversity areas in the Iberian Peninsula; c) analised the dynamics of *E. globulus* within the Natura 2000; d) performed spatial analyses to assess the conflicts between *E. globulus* plantations and high biodiversity areas; e) led paper writing, submission and revision.

Additional information:

The ‘appendices’ to this work can be accessed through the links below:

Appendix 1: https://www.dropbox.com/s/ijyjos3ndqogrh2/Appendix%20A%20-Tables%20A1-A4.xlsx?dl=0

Appendix 2: https://www.dropbox.com/s/jjgn2enqgwtezkl/Appendix%20B%20-%20Fig.%20B1-B3%20supplementary%20material.pdf?dl=0

1.1 Introduction

Plantations of *Eucalyptus globulus* Labill. expanded vertiginously since the mid-20th century in several regions of the world (Jacobs 1979; Potts et al. 2004), particularly in the Iberian Peninsula (Iberia), in Southwest Europe, including mainland Portugal and Spain. In Iberia, the area occupied by *E. globulus* increased about four-fold, from ca. 3,400 to 14,000 km^2**,** between 1970 and 2010, according to the national forest inventories. This expansion resulted mostly from a massive, uncontrolled proliferation of plantations in some regions, propelled mainly by small private landowners and pulp companies to a lesser extent, causing major landscape transformations (Calvo-Iglesias et al. 2006; Ruiz & Lopez 2010; Silva & Tomé 2016; Teixido et al. 2010). Over the last decades, *E. globulus* plantations acquired an increasing socioeconomic importance and became an important source of income for many landowners in some regions of both countries (Ruiz & Lopez 2010; Silva & Tomé 2016).

Iberia is a biodiversity hotspot and a key region for preserving many endangered species and habitats (Araújo et al. 2007; Myers et al. 2000; Underwood et al. 2009). The most comprehensive effort for nature conservation in Iberia was the establishment of the Natura 2000 network of protected areas, a pan-European initiative promoted by the European Union aimed at protecting the most endangered terrestrial and marine species and habitats (EEA 2017; Evans 2012). Nearly 20% of the terrestrial Natura 2000 is located in Iberia (ca. 156,000 km^2; Portugal: 20,000 km^2; Spain; 136,000 km^2), covering 26% of the Iberia territory. The terrestrial Natura 2000 in Iberia is composed of 1505 sites (Spain: 1,409; Portugal: 96) (EEA 2017). Iberia has also been referred to as a hotspot of High Nature Value farmlands (HNVf), corresponding to agricultural landscapes where low-intensity farming systems prevail, supporting high levels of biodiversity, including habitats and species of conservation concern (Halada et al. 2011; Paracchini et al. 2008). Despite the importance of HNVf for the conservation of the European Union natural capital, most HNVf currently occur outside protected areas, thus lacking any conservation status (EEA 2004).

The ecological impacts of planted forests have been subject of debate. Adequate planning and management may help to conserve biodiversity (Carnus et al. 2006; Hartley 2002), as shown in Brazil for some eucalypt plantations, through the preservation of a native understorey or native forests in their surroundings (Brockerhoff et al. 2013; Stallings 1990). However, in some Iberian regions, a massive, uncontrolled establishment of monospecific *E. globulus* plantations may cause harmful impacts on biodiversity, by the replacement of valuable habitats (Abelho & Graça 1996; Acácio et al. 2016; Pozo et al. 1998) and habitat fragmentation (Fahrig 2003; Taylor et al. 1993; Teixido et al. 2010). Ecological impacts of *E. globulus* plantations received much attention in recent years, particularly in Iberia (e.g. Calviño-Cancela 2013; Castro-Díez et al. 2012; Proença et al. 2010; Rodríguez-Suárez et al. 2011). Nonetheless, this is still a controversial topic as the conclusions are normally context-dependent (Poore & Fries 1985; Rejmánek & Richardson 2011).

Climate change is likely to have an impact in the conflicts between *E. globulus* plantations and high biodiversity areas. In fact, Iberia is expected to be one of the most responsive regions to climate change (Giorgi 2006). As a result, tree species in Iberia will likely experience considerable range shifts (Garzón et al. 2008), including alien tree species, which may pose new threats to biodiversity (Vicente et al. 2011). The extent of such range shifts may be determined by the magnitude of climate change (Butt et al. 2013; Klausmeyer & Shaw 2009). Despite the proliferation of *E. globulus* plantations in Iberia, to date, no objective assessment has been made regarding the potential conflicts between these plantations and high biodiversity areas.

The main goal of this study was to assess the potential conflicts between *E. globulus* plantations and high biodiversity areas in Iberia, namely sites designated for conservation within the Natura 2000 network and areas identified as HNVf. Specific objectives were: a) to analyse the distribution and temporal dynamics of *E. globulus* plantations inside and around the Natura 2000 network; b) to identify the main environmental factors determining the distribution of *E. globulus* plantations; c) to use a modelling approach to project the current and future potential ranges of *E. globulus* plantations, under different climate change scenarios; d) to identify current and future potential conflicts of *E. globulus* plantations with the Natura 2000 sites and HNVf. Drawing on these results, we discuss possible future implications for biodiversity in Iberia and suggest measures to mitigate conflicts and impacts.

1.2 Materials and Methods

1.2.1 Study area

The study area includes the mainland area of Portugal and Spain (Iberia), located in the southwest of Europe, covering around 580,000km^2. Iberia is located in the temperate zone, between ca. 36º00'N and 43º47'N. A Mediterranean climate regime predominates in Iberia, with dry and hot summers, contrasting with moist winters. Summer is hotter and dryer in the southern plateau and the Mediterranean coast, while it is cooler and wetter along the western and northern coasts due to the Atlantic influence. Overall, total annual precipitation increases from the southeast to the northwest, reaching maximum values in the northwest and north Atlantic coasts (AEMET/IM 2011).

1.2.2 Study species

Eucalyptus globulus Labill. (Myrtaceae) is an evergreen tree, up to 60 m height, native to Tasmania, Bass Strait Islands and southern Victoria (Potts et al. 2004). The species is cultivated in several regions worldwide (Jacobs 1979; Potts et al. 2004). In Iberia, it is mostly cultivated for the pulpwood and paper markets, coppiced every 10-12 years up to three rotations (Silva & Tomé 2016). In Portugal, *E. globulus* plantations cover ca. 8,500 km^2, the equivalent to ca. 9% of the country, mostly in Central and Northwest Portugal. In Spain, plantations cover ca. 5,900 km^2, concentrated in the southwest and the northern Atlantic shores (Fig. 1). Plantations are distributed across a climatic, geological and altitudinal gradient (Catry et al. 2015; Serrada et al. 2008), but greater densities of *E. globulus* plantations are found in the most productive regions (Tomé 2000).

1.2.3 Assessing the dynamics of *E. globulus* in the Natura 2000

The distribution and temporal dynamics of *E. globulus* plantations inside and around the Natura 2000 network were assessed in GIS software, using vectorial maps of the Natura 2000 (EEA 2017; Fig. 1) and vectorial land-cover maps for two time periods in each country: 1990 and 2007 for Portugal (DGT 2007; IGP 1990); 1996 and 2006 for Spain (MAPAMA 1996, 2006). The 1996 land-cover map of Spain does not discriminate the eucalypt species, and should include a residual occupation of *Eucalyptus camaldulensis* Dehnh. in the southwest of Spain.

We performed three analyses. First, we assessed the current occupation of *E. globulus* in the Natura 2000 sites (Fig. 1), and verified if *E. globulus* plantations were present at the time of sites' designation. Second, we assessed the temporal dynamics of *E. globulus* plantations inside the Natura 2000 network between the time periods referred above. We restricted this analysis to the sites that were designated as protected areas before the first land-cover map for each country, i.e. we retained only the Portuguese sites designated before 1990 and the Spanish sites designated before 1996, to ensure that any expansion or reduction of *E. globulus* plantations inside the sites occurred after their designation. Additionally, among these sites, we retained only the sites that hosted *E. globulus* plantations in any of the two land-cover maps, in order to register both the expansion and the reduction of plantations in the sites along this period. Twenty-six sites fulfilled these criteria (Portugal: 12; Spain: 14), including Special Protection Areas (Birds Directive) designated since 1987 in Spain and 1988 in Portugal, that were later assimilated by the Natura 2000 designation. Third, we assessed the temporal dynamics of *E. globulus* plantations within a buffer of 1 km around the Natura 2000 sites.

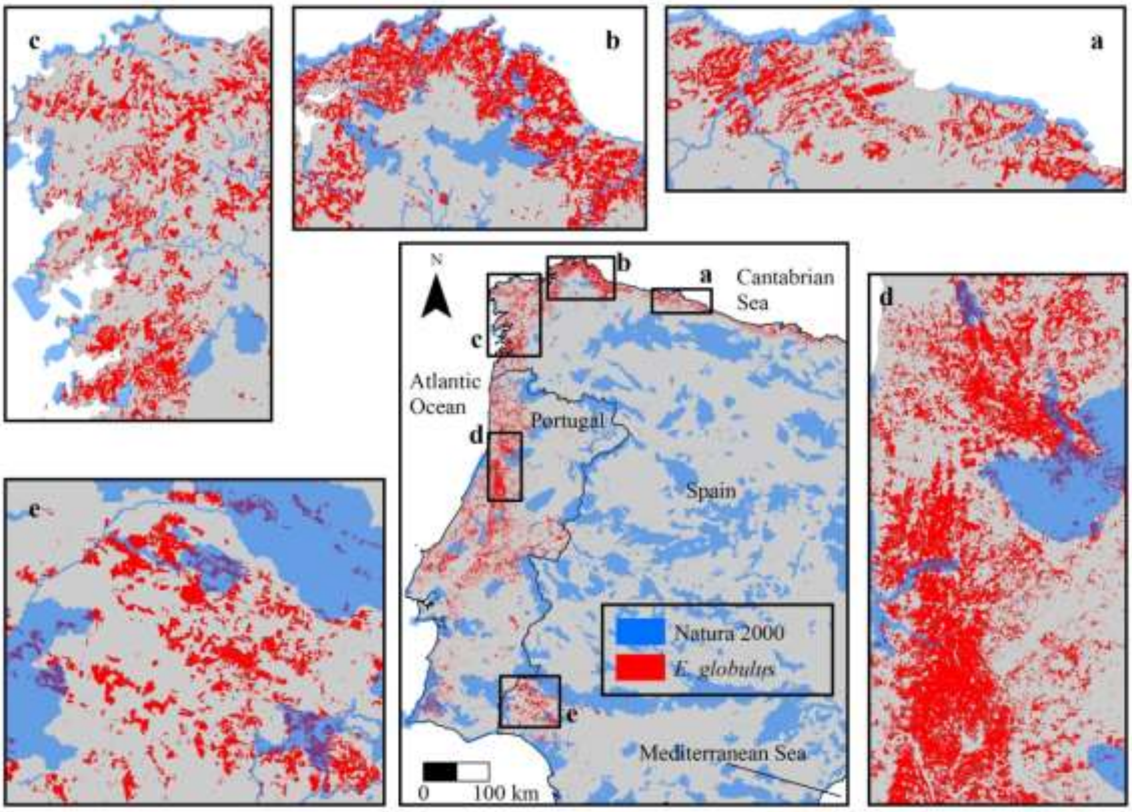

Fig. 1 - Distribution of *Eucalyptus globulus* and the terrestrial Natura 2000 in Iberia. Zoom-in on representative regions featuring high density of plantations

1.2.4 Species distribution models

1.2.4.1 Modelling framework and assumptions

We calibrated *species distribution models* (SDM) to estimate the potential range of *E. globulus* plantations under current and future environmental conditions (IPCC 2014) using the R package *biomod2* (Thuiller et al. 2016). As the maps of potential range include levels of environmental suitability for *E. globulus* plantations, we assumed that locations exhibiting higher suitability have higher chances of accommodating more plantations, according to what we can actually observe in Iberia (Silva & Tomé 2016; Tomé 2000). This is a fair assumption considering that: most rural area in Iberia is private property (Beires et al. 2013; MAGRAMA 2012); species distribution is mostly determined by human-made decisions, because we are dealing with a cultivated species; within the suitable area for its cultivation, *E. globulus* has been the preferred forestry species by landowners. SDM are often criticized because important explanatory factors are omitted, such as biotic interactions, evolutionary changes and dispersal characteristics (Heikkinen et al. 2006; Pearson & Dawson 2003). In this study, those limitations are minimized as: the data used to build the models were mostly from planted areas; we are dealing with a cultivated species, whose distribution is not dependent on its natural dispersal ability; the species is normally established under favourable conditions, with reduced competition with other species.

1.2.4.2 Presence-absence data

Data on *E. globulus* distribution was gathered from vectorial land-cover maps of Portugal (DGT 2007) and Spain (MAPAMA 2006). Presence of *E. globulus* was recorded at a resolution of 10x10 km, using a regular grid of cells covering Iberia, totalling 6036 cells. Presence aimed to capture the suitable areas for the species cultivation (not the species' ecological niche), since we are dealing with plantations. Therefore, *presence* was considered in any cell with a coverage ≥10% of pure (monospecific) *E. globulus* stands, because greater coverage of plantations is normally found in the most productive areas (Silva & Tomé 2016). The existence of pure stands should normally indicate that plantations are actively managed, thus located in productive locations, otherwise they would likely convert into mixed stands (Moreira et al. 2009). Using these criteria, presence was recorded in 334 cells. The same number of pseudo-absences (n=334) was randomly selected among the *non-presence* cells. To avoid the model being skewed due to the pseudo-absence selection, the full presence set was used together with three sets of pseudo-absences randomly generated using *biomod2* function, weighting presences and pseudo-absences equally during the calculation (prevalence=0.5; Wisz & Guisan 2009).

1.2.4.3 Environmental variables as SDM predictors

A set of environmental variables was used to model the current potential range of *E. globulus* plantations in Iberia, including bioclimatic (Fick & Hijmans 2017) and geological variables (OneGeology 2018). Variables were chosen based on the existing knowledge about the environmental conditions determining *E. globulus* distribution (Jacobs 1979; Kirkpatrick 1975; Ribeiro & Tomé 2000). Also, the selected bioclimatic variables allowed forecasting future climate data (see section 1.2.4.5). Only variables presenting pairwise *Spearman* correlations <0.7 were retained. When variables were correlated, we chose the one with the most direct ecological impact (based on expert knowledge) on plant species distribution (Guisan & Thuiller 2005). This analysis yielded a final set of 14 variables: seven bioclimatic and seven geological variables (Table 1). The importance of each environmental variable was estimated for the ensemble model prediction (Thuiller et al. 2016).

1.2.4.4 Model calibration and evaluation

Model calibration and evaluation were performed using the ten available modelling algorithms of the *biomod2* package (for more details see *biomod2* help files and vignettes) and the selected set of 14 environmental variables. Each individual model was calibrated using 80% of available data. The area under the curve (AUC) was then calculated on the 20% of remaining data. The final ensemble model was obtained by the predictions of all models with AUC above 0.7, using the Mean (all) consensus method (see Marmion et al. 2009).

Model projection was reclassified into a probability ramp using a threshold maximizing the percentage of presences and absences correctly predicted (i.e. the probability where sensitivity = specificity; Liu et al. 2005) to classify absences, and then a probabilistic ramp from the threshold to the maximum predicted value using the "filtROC" function available in *biomod2* (for more details see *biomod2* help files and vignettes). The probability ramp can be assumed as a degree of environmental suitability for the establishment of *E. globulus* plantations.

Table 1 - Original sets of bioclimatic and geological variables considered to model the potential range of *Eucalyptus globulus* plantations. Asterisks indicate uncorrelated variables selected for modelling calibration. Sources: bioclimatic variables (Fick & Hijmans 2017); geological variables (OneGeology 2018)

Type of variables	Variables
Bioclimatic	Annual Mean Temperature Mean Diurnal Range Isothermality* Temperature Seasonality* Maximum temperature of the warmest month Minimum temperature of the coldest month* Temperature annual range Mean temperature of the wettest quarter* Mean temperature of the driest quarter Mean temperature of the warmest quarter Mean temperature of the coldest quarter Annual Precipitation* Precipitation of the wettest month Precipitation of the driest month Precipitation seasonality* Precipitation of the wettest quarter Precipitation of the driest quarter Precipitation of the warmest quarter* Precipitation of the coldest quarter
Geological	Felsic and intermediate igneous rocks* Mafic igneous rocks* Non foliated metamorphic rocks* Foliated metamorphic rocks* Carbonate sedimentary rocks* Water*

1.2.4.5 Projecting current and future ranges of *E. globulus* plantations

After calibration, we projected the current and future potential ranges of *E. globulus* plantations in Iberia. The potential range was classified according to five levels of environmental suitability for *E. globulus* plantations: 1: not suitable (<1%); 2: very low suitability (1-25%); 3: low suitability (26-50%); 4: high suitability (51-75%); 5: very high suitability (76-100%). To project the models of *E. globulus* plantations range under future climatic conditions, we chose two plausible and accessible socio-economic scenarios from the Intergovernmental Panel on Climate Change (IPCC 2014) for the years 2050 and 2070, corresponding to two contrasting representative concentration (of greenhouse gas) pathways (RCP): RCP2.6 and RCP8.5. The RCP2.6 assumes a global development following sustainable locally oriented pathways, with lower rates of global population growth and yielding moderate increases in temperature, between 0.3ºC and 1.7ºC in the late-21th century. The RCP8.5 assumes a very rapid economic growth with increasing globalisation, a balanced requirement of different fossil and non-fossil energy sources, leading to a great increase in temperature, ranging between 2.6ºC and 4.8ºC in the late-21th century (IPCC 2014; Moss et al. 2010). The intermediate year of 2050 was included because the RCP2.6 considers an inversion on greenhouse gas concentration around this period. Only bioclimatic variables were used in the models. For each bioclimatic variable, under each RCP/year, a consensus map (average values) was produced using all available general circulation models (14 in common for both RCPs; Fick & Hijmans 2017). As a result, this study presents five projections: 1) current potential range; future potential ranges under the RCP2.6 for the years 2) 2050 and 3) 2070; future potential ranges under the RCP8.5 for the years 4) 2050 and 5) 2070.

1.2.5 Rating conflicts with biodiversity areas

The distribution of HNVf was produced by upscaling the map from Paracchini et al. (2008) to a 10x10 km grid of cells. A "conflict" was defined as the co-existence, in the same 10x10 km cell, of *E. globulus* plantations and biodiversity areas, namely Natura 2000 and HNVf sites. Conflicts were rated based on the different combinations between the level of environmental suitability for *E. globulus* plantations and the level of importance of biodiversity areas in each cell, namely the number of Natura 2000 sites in each cell (no sites: less important biodiversity; 1-2 sites: important biodiversity; ≥3 sites: very important biodiversity) and, for HNVf, in conformity with the criteria from Paracchini et al. (2008), the likelihood of each grid cell to exhibit high nature value (0%: less important biodiversity; 1-50%: important biodiversity; >50%: very important biodiversity) (Table 2).

Table 2 - Rating of the conflicts between *Eucalyptus globulus* plantations and biodiversity areas

Levels of suitability for *E. globulus* plantations	Levels of biodiversity importance		
	Very important biodiversity	Important biodiversity	Less important biodiversity
Very high (>75%)	Highest concern [7]	Probable conflict with high concern [6]	No conflict but suitable conditions for plantations [2]
High (51-75%)	Conflict possible with very high concern [5]	Conflict possible with high concern [4]	
Low (26-50%)			
Very low (1-25%)	Lowest concern [3]		
Unsuitable (<1%)	No conflict [1]		

The rating of conflicts for the Natura 2000 and HNVf was merged in a single table for convenience, since the analyses were conducted separately. Numbers between square brackets (from 1 to 7) find correspondence with the levels of conflict in Fig. 4 and 5

1.3 Results

1.3.1 Is *E. globulus* expanding inside and around the Natura 2000 network?

In 2006/7 (Spain/Portugal), nearly 10% (1,463 km^2) of the area of *E. globulus* plantations in Iberia was distributed across 235 areas currently designated as Natura 2000 (Portugal: 1,026 km^2; Spain: 437 km^2). On average, *E. globulus* plantations covered 5.8% of these areas (n=235), reaching more than 20% cover in 21 areas, and more than 50% in four areas. There were at least 150 Natura 2000 sites which hosted *E. globulus* plantations at the time of designation, 16 of which exhibited a cover higher than 20%.

The dynamics of *E. globulus* plantations inside Natura 2000 after site's designation was assessed on 26 sites (see also section 1.2.3). Overall, between 1990/96 (Portugal/Spain) and 2006/7, *E. globulus* cover decreased ca. 37% (from 343 to 215 km^2), even though it increased in 19 out of the 26 sites (73%). A thorough analysis of this result revealed that the overall reduction was mainly influenced by three sites in southwest Spain (Andalusia). In fact, without these three sites, *E. globulus* cover would have increased 34% (from 105 to 141 km^2). At a country-level, in Portugal (n=12), the area occupied by *E. globulus* increased 48% (from 29 km^2 to 43 km^2; +0.8 km^2 year^{-1}), while in Spain (n=14) it decreased 45% (314 km^2 to 172 km^2; -14 km^2 year^{-1}). Again, without the three sites in southwest Spain, the area occupied by *E. globulus* in the Spanish sites would have increased 32% (from 75 to 99 km^2; +2.4 km^2 year^{-1}). Note that the reduction of *E. globulus* plantations in southwest Spain can also be partly explained by the fact that, in the first land-cover map (MAPAMA 1996), an undefined amount of *E. camaldulensis* plantations was accounted as *E. globulus* plantations (see section 1.2.3).

Around the areas currently designated as Natura 2000 (1 km buffer), the *E. globulus* cover increased 71% between 1990/6 and 2006/7 (from 853 to 1,457 km^2). Around the Portuguese sites, the *E. globulus* cover increased 46% (from 388 to 567 km^2), while around the Spanish sites it increased 69% (from 589 to 995 km^2).

1.3.2 What is the current potential range of *E. globulus* plantations?

Overall, the goodness of the ensemble model denoted an excellent accuracy, with an AUC of 0.964. The current potential range of *E. globulus* plantations was found to be mainly determined by two bioclimatic variables: the *minimum temperature of the coldest month* (44.8% importance; positive effect); and, the *temperature seasonality* (10.5% importance; negative effect). The remaining variables had an importance below 10% (see the importance of all variables in Appendix A, Table A1; see model plots in Appendix B, Fig. B1 and B2).

The current potential range of *E. globulus* plantations extends over 106,800 km^2 along most of the Atlantic shore, corresponding to ca. 18% of Iberia. The species' range is wider in southwest Iberia, reaching ca. 200 km from the coastline, and progressively narrows towards the north, ending in a thin coastal strip along the northern shores (Fig. 2). Most (72%) of the "suitable range" exhibits *high* (28%) or *very high* (44%) suitability. The lowest suitability for *E. globulus* plantations is observed in southwest Iberia and along the inner boundaries of the suitable range. In 2006/7, *E. globulus* plantations occupied 13.4% of the current potential range, according to the following distribution: 63% in *very high suitability* areas; 22% in *high suitability* areas; 8% in *low suitability* areas; 3% in *very low suitability* areas; 4% in *unsuitable* areas (Fig. 2). Similar patterns of distribution were found in the two Iberian countries.

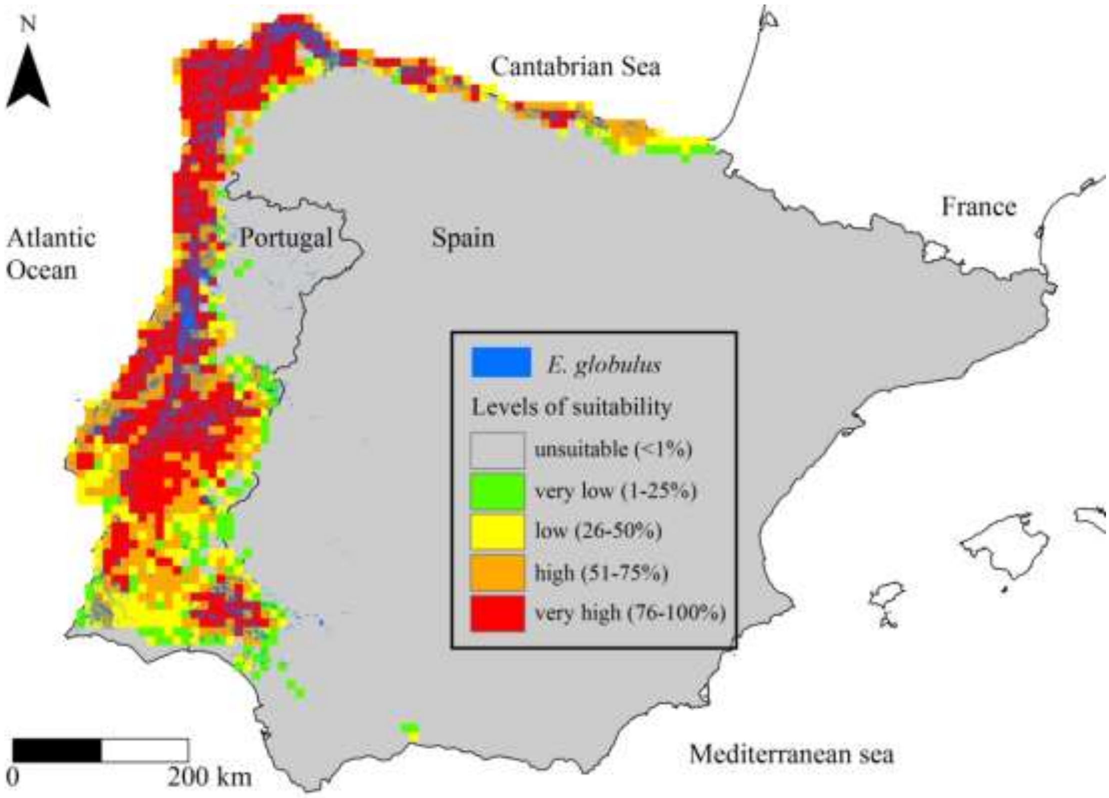

Fig. 2 - Distribution of *Eucalyptus globulus* plantations (blue) over the current potential range. The potential range includes levels of environmental suitability for plantations

1.3.3 How will *E. globulus* plantations be distributed in the future?

In 2070, the suitable range of *E. globulus* plantations is expected to decrease to 92% and 55% of the current extent under the RCP2.6 and the RCP8.5, respectively. The loss of suitable range is expected to occur mostly in southwest Iberia. This loss is much more expressive under the RCP8.5, where large contiguous clumps of suitable range are lost in each time frame (Fig. 3).

Both RCP scenarios show a gain of suitable range in the northern inner boundaries, which is greater under the RCP8.5 (see Appendix B: Fig. B3). In the year 2070, under the RCP8.5, the suitable range begins to disappear along the shore of the Cantabrian Sea. Within the suitable range, the proportion of *very high* suitability areas increases from 44% (47,200 km^2) on the current potential range, to 69% (67,900 km^2) and 62% (37,000 km^2) in 2070 under the RCP2.6 and the RCP8.5, respectively (see Appendix A: Table A2). Under both RCPs, in 2070, the regions identified as being of major potential conflict are the northwest of Portugal (and Central Portugal under the RCP2.6), and the northwest and northern shore of Spain.

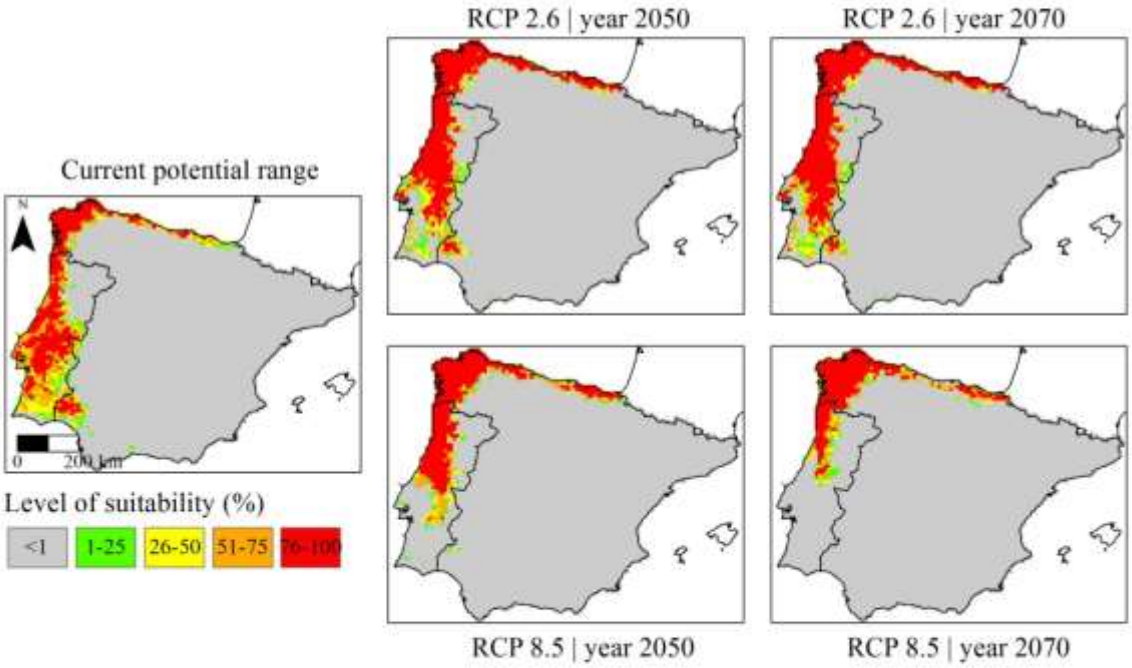

Fig. 3 - Current potential range of *Eucalyptus globulus* plantations and future potential ranges for the years 2050 and 2070 under the RCP2.6 and the RCP8.5 scenarios. The potential range is classified according to levels of environmental suitability for plantations

1.3.4 Where are conflicts with Natura 2000 expected to occur?

According to our projections, in 2070, the extent of *conflict* between *E. globulus* plantations and Natura 2000 areas, i.e. the number of cells combining the existence of Natura 2000 sites and suitable conditions for plantations, is expected to decrease 13.7% under the RCP2.6 and 41.7% under the RCP8.5 compared to the current potential range. Likewise, the number of Natura 2000 sites located within the suitable range of *E. globulus* plantations will decrease from 254 to 244 sites under the RCP2.6, and to 196 sites under the RCP8.5. These results depict the balance between the number of Natura 2000 sites maintained, lost and added by a shifting range until the year 2070, totalling, respectively, 215, 39 and 29 sites under the RCP2.6, and 142, 112 and 54 sites under the RCP8.5.

In the current potential range, the most concerning *conflict areas*, classified as *highest concern* (level 7; see Table 2) and *probable conflict with high concern* (level 6), cover 2,590 km^2 (25% of the range). Under the RCP2.6, the extent of level 6-7 conflict areas increases to 4,010 km^2 in 2070 (41%), while under the RCP8.5 it increases to 3,180 km^2 in 2050 (43%), followed by a decrease to 2,470 km^2 in 2070 (42%; see also Appendix A: Table A3). Under both RCPs, level 6-7 conflict areas concentrate along the northern shore and northwest Iberia. There is also an expansion of level 6-7 conflict areas in the northern half of Portugal, which seems to cease before 2070 under the RCP8.5 (Fig. 4).

1.3.5 Where are conflicts with High Nature Value farmlands expected to occur?

Between the current and the 2070 projection, the extent of the *conflict area* with HNVf, i.e. the number of cells featuring HNVf and suitable conditions for *E. globulus* plantations, is expected to decrease 8.2% under the RCP2.6 and 54% under the RCP8.5. In the current potential range, the areas exhibiting the higher levels of conflict, *highest concern* (level 7; see Table 2) and *probable conflict with high concern* (level 6), cover 1,710 km^2 (16% of the suitable range). Under the RCP2.6, the extent of level 6-7 conflict areas increases to 2,660 km^2 in 2070 (28%), while under the RCP8.5 it decreases to 1,330 km^2 in 2070, even though their representativeness increases to 22% of the suitable range (see also Appendix A: Table A4). Under both RCPs, there is an expansion of level 6-7 conflict areas along the northern

Spanish shore, in the northwest of Spain and, particularly under the RCP2.6, in Central Portugal and the northern half of Portugal (Fig. 5).

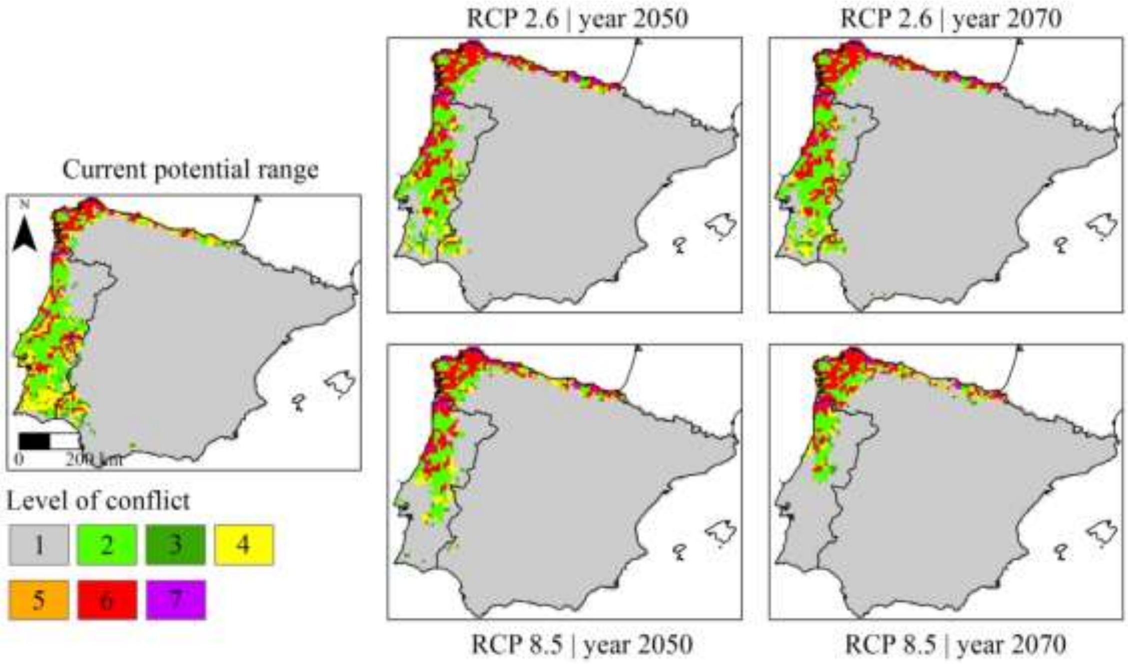

Fig. 4 - Levels of conflict between *Eucalyptus globulus* plantations and Natura 2000 sites for current times and for the years 2050 and 2070 under the RCP2.6 and the RCP8.5 scenarios. The levels of conflict correspond to: 1: no conflict; 2: no conflict but suitable conditions for plantations; 3: lowest concern; 4: conflict possible with high concern; 5: conflict possible with very high concern; 6: probable conflict with high concern; 7: probable conflict with very high concern (see Table 2)

1.4 Discussion

1.4.1 Dynamics of *E. globulus* plantations in the Natura 2000

Eucalyptus globulus plantations are currently spread across 235 Natura 2000 sites, occupying a considerable area in some sites. Nearly 70% of the *E. globulus* cover inside the Natura 2000 network was found inside Portuguese sites, even though the extent of the terrestrial Natura 2000 is ca. seven times greater in Spain. This disparity between countries may be partly explained by the fact that: Portugal is more than five times smaller than Spain; the total area of *E. globulus* plantations is nearly 1.4 larger in Portugal; the current potential range of *E. globulus* plantations covers most of Portugal, as well as most of the Portuguese Natura 2000 sites, in contrast to a small fraction of Spain. Both countries have restrictive regulations on protected areas. Therefore, it is likely that *E. globulus* plantations preceded these protected areas. In fact, we found that *E. globulus* plantations were already present in most of the Natura 2000 sites at the time of designation as a protected area, which is symptomatic of the ubiquity of these plantations. On the other hand, it may suggest that *E. globulus* plantations can co-exist with areas of high biodiversity value, even though there are reports of negative impacts associated with *E. globulus* plantations inside protected areas (e.g. Teixido et al. 2010).

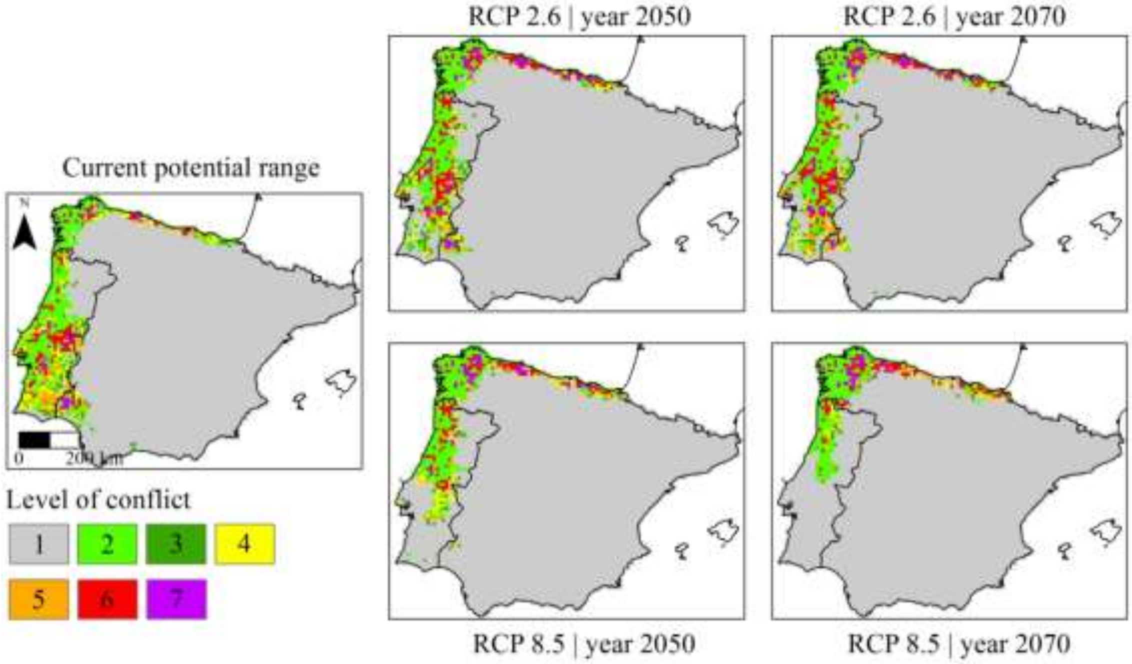

Fig. 5 - Levels of conflict between *Eucalyptus globulus* plantations and High Nature Value farmlands for current times and for the years 2050 and 2070 under the RCP2.6 and the RCP8.5 scenarios. The levels of conflict correspond to: 1: no conflict; 2: no conflict but suitable conditions for plantations; 3: lowest concern; 4: conflict possible with high concern; 5: conflict possible with very high concern; 6: probable conflict with high concern; 7: probable conflict with very high concern (see Table 2)

In a small sample of Natura 2000 sites, we found an overall reduction of the coverage of *E. globulus* plantations after their designation as protected areas. This reduction was most expressive in southwest Spain, likely as a result of an inversion of regional forestry policies that led to the removal of eucalypt plantations (Álvarez 2011; Ovando et al. 2009). However, *E. globulus* plantations expanded in a small number of sites, both in Portugal and Spain. In Portugal, a governmental report acknowledged that *E. globulus* plantations replaced important habitats, being one of the threats to some habitats and species (ICNB 2008). Such land-cover changes inside the Natura 2000 network most probably eluded the prevailing regulations or the site's management plan when existent, suggesting a lack of supervision and law enforcement. Around the Natura 2000 sites, there was a substantial expansion of *E. globulus* plantations. On one hand, it suggests that the conservation status of the Natura 2000 sites has prevented a higher expansion of plantations. On the other hand, such an expansion suggests that the surroundings of the Natura 2000 sites have been neglected.

1.4.2 The current potential range of *E. globulus* plantations

Temperature-related variables were determined to explain the current potential range of *E. globulus* plantations, especially the *minimum temperature of the coldest month*, reflecting the sensitivity of small *E. globulus* seedlings to frost (Jacobs 1979; Kirkpatrick 1975). The low tolerance of *E. globulus* to wide variations in temperature should explain the proliferation of plantations along the Atlantic shore, where temperature extremes are attenuated (AEMET/IM 2011). Areas of *high* and *very high suitability* for *E. globulus* plantations predominate in most of the current potential range. However, there are likely some artifices, particularly in the *very high suitability* of some regions in the southern half of Portugal and in the southwest of Spain. This artifice is caused by the abundance of *E. globulus* plantations in these regions characterized by a lower productivity (Álvarez 2011; Ribeiro & Tomé 2000), suggesting that *E. globulus* plantations may also expand in disadvantaged areas. There are also a few scattered plantations outside the potential range, probably occupying the limits of the species' tolerance in terms of rainfall shortage and temperature seasonality. In fact, in the southwest of Spain, *E. globulus* plantations overlap the range of *Eucalyptus camaldulensis* Dehnh. (MAPAMA 2006), a species with greater tolerance to drought and extreme temperatures (Jacobs 1979).

1.4.3 Future potential ranges of *E. globulus* plantations

Eucalyptus globulus plantations are expected to experience considerable range shifts due to climate change, like many native tree species (Garzón et al. 2008). The range shift will be determined by the magnitude of climate change, as observed in other studies (Butt et al. 2013; Klausmeyer & Shaw 2009). Under the RCP2.6, the loss of suitable range is practically negligible, and even tends to recover following the mid-century inversion on greenhouse gas concentration. In contrast, under the RCP8.5, the suitable range will be confined to nearly half of the original extent in 2070, with the loss of suitable range progressing northwards along this period. Both scenarios suggest, with different magnitudes, a poleward shifting of *E. globulus* plantations, as predicted for many other plants and animals (Harrison et al. 2006; Hickling et al. 2006). In Iberia, this *migration* is inevitably blocked by the Cantabrian Sea, resulting in a range contraction.

The contraction of the suitable range of *E. globulus* plantations does not necessarily imply a reduction of the area occupied by *E. globulus*. In fact, in both climate change scenarios, the range contraction may be counterbalanced for at least four reasons. First, new suitable areas for *E. globulus* plantations will likely emerge in the northern inner borders of the range, where there may be greater potential in terms of space for accommodating new plantations. Second, the loss of suitable range does not necessarily mean that *E. globulus* plantations will be removed from the areas that became unsuitable for cultivation. Older plantations in these areas will probably become abandoned (as currently happens), with no productive or conservation utility. Third, market demands, following the decrease of productive plantations due to the shrinking range, may encourage the establishment of new plantations, including in areas of lower productivity, as we see today in Iberia. Finally, many areas currently exhibiting lower suitability for *E. globulus* plantations will become of *very high suitability*, meaning that the cultivation of *E. globulus* plantations will become much more appealing in these areas, encouraging the establishment of new plantations.

Nevertheless, the future projections omit several factors, particularly anthropic drivers, which may help to shape the future distribution of *E. globulus* plantations. Governments in both countries have been developing legislation to control and regulate the establishment of *E. globulus* plantations. We cannot forecast future policies (and their efficiency), socioeconomic changes or market demands, which may severely limit, revert or perhaps even encourage the expansion of *E. globulus* plantations, resulting in more or less distinct scenarios than the ones we present.

1.4.4 Conflicts with high biodiversity areas

The reduction of the extent of conflict areas with both Natura 2000 and HNVf is a direct result of the contraction of the suitable range of *E. globulus* plantations. Despite range contraction, the suitability for plantations is expected to improve (to *very high suitability*) on a larger portion of the suitable range, leading to an aggravation of conflicts. These trends are shared by the two types of biodiversity areas because both are fairly distributed across the potential range of *E. globulus* plantations, and because they partly overlap, since some HNVf integrate with Natura 2000 (Halada et al. 2011; Paracchini et al. 2008). In the case of Natura 2000, under both climate change scenarios, northwest Iberia (including the northwest of Spain and the northwest of Portugal) is the region where the most serious conflicts are expected. However, under the RCP2.6, other regions were found to be particularly concerning, namely the northern Spanish coast and Central Portugal. Areas of conflict between *E. globulus* plantations and HNVf are more scattered but, under both RCPs, relatively large extensions of these areas are expected to be found in northwest Iberia and the northern Spanish coast. Under the RCP2.6, in particular, the highest number of conflicts with HNVf is also expected to occur in Central Portugal.

There is a trade-off between conflict extent and conflict level on the two climate change scenarios. On one hand, under the RCP2.6, there are more high biodiversity areas under potential conflict, because the suitable range of *E. globulus* plantations is wider. On the other hand, the materialization of conflicts, and their exacerbation, is more likely under the RCP8.5, because *E. globulus* plantations will be confined to a smaller area, causing a greater concentration of plantations. Therefore, both scenarios of climate change are potentially harmful for both types of biodiversity areas following the possible range dynamics of *E. globulus* plantations. Apart from these considerations, it should be consensual that the RCP8.5 is the worst of these scenarios, because climate change itself is a major threat to biodiversity (Pacifici et al. 2017; Thomas et al. 2004). It is also worth mentioning that, without additional efforts to constrain greenhouse gas emissions, the RCP8.5 is considered to be a more likely scenario (IPCC 2014).

1.4.5 Mitigating the conflicts and impacts

The socioeconomic importance of *E. globulus* in both Iberian countries requires efforts in reconciling *E. globulus* plantations with nature conservation goals. It is known that adequate planning and management can alleviate the potential negative impacts of planted forests (Brockerhoff et al. 2008; Fischer et al. 2006; Hartley 2002), as shown in some Brazilian eucalypt plantations, by maintaining a native understorey or preserving native forests in the surrounding landscape (Brockerhoff et al. 2013; Stallings 1990). However, ecological benefits will hardly be achieved with the uncontrolled, massive expansion of mono-specific *E. globulus* plantations in some Iberian regions. Governments in both Iberian countries have been implementing legislation to control and regulate the expansion of *E. globulus* plantations, but it has proven to be flawed and inadequate to address local realities. This study shows that particular attention should be devoted to the potential impacts on high biodiversity areas, and helps identify the areas of greatest concern in current and future times.

The proliferation of *E. globulus* plantations around Natura 2000 sites is of major concern due to the potential of hindering connectivity between sites. Management of *E. globulus* plantations towards the preservation of a native understorey would likely enhance landscape connectivity (Calviño-Cancela et al. 2012). HNVf can play a key role connecting protected areas. HNVf require particular attention because, apart from other threats such as wildfires, socioeconomic changes (e.g. rural exodus, agriculture intensification) are leading to their decline and most of them lack conservation status (Jongman 2002; Plieninger et al. 2006; Stoate et al. 2009). Besides, their mapping, the records of their biodiversity and their monitoring are still deficient (EEA 2004; Lomba et al. 2014). The efforts to preserve these agricultural systems should prioritize the ones which may enhance the connectivity between protected areas and promote landscape heterogeneity. In this regard, protected areas like the Natura 2000 sites should feature a surrounding safety zone with specific planning and regulation, particularly in relation to landscape planning, land-use and forest management to promote species movement and gene flow, and prevent harmful impacts on vulnerable habitats and species, including wildfires and alien plant invasions.

Several authors denounced the fragilities of the Natura 2000 network, such as the deficient planning, management and monitoring, insufficient funding, ineffective law enforcement, disaffection of landowners and conflict of interests with landowners (Apostolopoulou & Pantis 2009; Fuentes et al. 2011; Geitzenauer et al. 2016; Wätzold et al. 2010). In Iberia, the overwhelming predominance of a highly fragmented private property, particularly in the regions where *E. globulus* plantations find the best conditions for cultivation, is an overarching constraint to overcome these deficiencies. Therefore, landowners are key stakeholders for conservation efforts (Carvalho-Ribeiro et al. 2010; Fuentes et al. 2011).

In this study we considered high biodiversity areas to be static. However, like *E. globulus* plantations, many endangered species in the Natura 2000 may lose suitable climate in current locations (Araújo et al. 2011), and will be forced to migrate, on their own or by human assistance, also towards the north and higher altitudes (Hickling et al. 2006; Lenoir et al. 2008; Pereira et al. 2010). It is thus fair and wise to foresee the need to create new protected areas or expand the existing ones, particularly in the northern regions of Iberia. However, future conservation efforts may become jeopardised by the current occupation of *E. globulus* plantations, but also by the possible expansion of plantations in the absence of effective law enforcement (Santos et al. 2016). It is thus urgent to prevent and, preferably to revert, the expansion of *E. globulus* in the regions that may form future refuges for the Iberian biodiversity.

1.5 Conclusions

The current potential range of *E. globulus* plantations extends over 18% of Iberia, mostly along the Atlantic shore, being strongly influenced by the *minimum temperature of the coldest month*. *Eucalyptus globulus* plantations are currently widespread within the Natura 2000. Many Natura 2000 sites already hosted *E. globulus* plantations at the time of designation. However, we found that *E. globulus* plantations have expanded inside some Natura 2000 sites. Moreover, *E. globulus* plantations have been proliferating around Natura 2000 sites. We expect a northward contraction of the suitable range of *E. globulus* plantations, much more expressively under the worst climatic scenario (RCP8.5), as a result of the loss of suitable range in the southwest. Despite the contraction of the suitable range, conflicts with both Natura 2000 and HNVf are expected to worsen under both climate change scenarios. In fact, for each climate change scenario, in each year (2050 and 2070), the extent of the areas exhibiting the most concerning conflicts will increase either in absolute terms (total surface), relative terms (fraction of the total suitable range) or in both. The potential expansion of *E. globulus* plantations in the absence of effective law enforcement may seriously jeopardise future conservation efforts. This study identifies the areas of highest concern, where corrective and preventive measures are more critical.

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Chapter 2: Google Street View as an alternative method to car surveys in large-scale vegetation assessments

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Author contribution:

a) helped conceiving the experimental design; b) collaborated in the predecessor study (Catry et al. 2015) that originated this study, by helping in the experimental design, in field work and manuscript writing; c) performed some statistical analyses; d) led paper writing, submission and revisions.

Additional information:

In this paper, we did not spend much efforts discussing the influence of regional-scale factors on the natural establishment of *E. globulus* because they were discussed in a previous study using car surveys (Catry et al. 2015). As stated in the current study, ‘both methods [car surveys and Google Street View] produced similar models of plant abundance, selecting the same explanatory variables, in the same hierarchical order of importance and depicting a similar influence on plant abundance’. Therefore, discussing the effect of those factors would be redundant.

The ‘online supplementary material’ (OSM) associated to this work can be found in the following link: https://www.dropbox.com/s/rg8so0ch6r1oacl/ESM1-4.pdf?dl=0

2.1 Introduction

Roads are an ubiquitous component of many landscapes with important ecological impacts, such as promoting the dispersal of alien invasive plants (Forman & Alexander 1998; Trombulak & Frissell 2000). Alien plants are plants introduced by humans outside the native range, being regarded as invasive when generate reproductive offspring at considerable distances from the parent plants (Richardson et al. 2000). Roads may act as corridors for seed dispersal by means such as drainage (Wilcox 1989) or vehicles (Taylor et al. 2012; von der Lippe et al. 2013), while roadsides may provide favorable conditions (e.g. recurrent disturbance, more light and water) for the establishment and spread of alien invasive plants (Christen & Matlack 2006; Parendes & Jones 2000). Some may also invade nearby areas (Amor & Stevens 1976; Gelbard & Belnap 2003; Hansen & Clevenger 2005), becoming more problematic from a conservation perspective. Alien invasive plants may have important environmental and economical impacts (Pimentel et al. 2005; Pyšek et al. 2009)

Road surveys, conducted by car, are a common method for monitoring and assessing the distribution of alien invasive plants (Abella et al. 2009; McAvoy et al. 2012; Milton & Dean 1998). Car surveys (CS) focus on invasion-prone areas for many species, i.e. roadsides and nearby areas, that would likely be misrepresented in random systematic sampling (Shuster et al. 2005), such as forest national inventories (Olsen & Schreuder 1997; Tomppo et al. 2010). Despite being spatially biased, CS may provide similar results as field-based random surveys considering the probability of occurrence of invasive species within different land covers (Shuster et al. 2005), and may still produce fairly accurate predictive maps of species distribution (Kadmon et al. 2004). One of the main advantages of CS is to allow covering larger areas in shorter periods with fewer resources, when compared to on-foot surveys (Catry et al. 2015; Shuster et al. 2005). However, it may still require considerable resources (car, global positioning system [GPS] equipment, driver and one or more observers) and budget (fuel, accommodation). Besides, it has the inherent dangers of driving while conducting the survey.

Google Street View (GSV) has been suggested as a cost-effective alternative for the detection of alien invasive plants along roads (Mazerolle & Blaney 2010; Visser et al. 2014). It is a free-access web technology featured in Google Maps and Google Earth where users may experience a virtual travel along roads using georeferenced panoramic photographs taken with roughly regular short distances (max. 20 meters), allowing a contiguous observation along the road (Hoelzl & Marie 2014). Imagery is obtained mostly through car journeys, using special high resolution cameras on the roof of adapted cars (Anguelov et al. 2010). Google Street View has an extensive coverage in several countries (Google 2015), including rural areas and marginal roads, as well as different years and seasons.

Google Street View was tested as a survey method in the study of animal species, namely the pine processionary moth in France (Rousselet et al. 2013) and vulture species in Spain (Olea & Mateo-Tomás 2013), both studies revealing a positive performance of GSV when compared to field surveys. As to plant ecology applications, Mazerolle and Blaney (2010) conducted a preliminary test using GSV for the detection of alien invasive plants in Canada. Results were promising with several species being easily detectable, even though some known colonies were not detected.

In 2014, Catry et al. (2015) conducted a CS throughout Portugal aiming at assessing the abundance of *Eucalyptus globulus* Labill. wildlings established from plantations on adjacent roadsides. Surveyed areas were later revisited using GSV (where available) and results from both methods were combined by choosing the higher wildling abundance registered at each location, as a way to better reflect the site potential for wildling establishment and minimize the influence of temporal factors such as roadside maintenance. As a result, 16% of the CS observations were replaced by GSV data.

In the present study we compare the results obtained by each method individually (CS vs. GSV), using wildling abundance data collected at the same locations, in order to test if we could replicate the results from this countrywide survey conducted by car, using GSV as a remote sensing tool. Our main goal was to assess the efficiency of GSV as novel plant survey method by comparing its results with a conventional method in plant ecology. Several environmental variables were collected to produce models of wildling abundance using boosted regression trees (BRT). In particular, this paper aims to: a) compare the results from both methods in terms of estimated plant abundance (section 2.3.1); b) compare the two method-specific models of plant abundance in terms of performance (explained deviance) and selected explanatory variables (section 2.3.2); c) verify if a set of variables collected exclusively with GSV improves model performance (section 2.3.2); d) compare the time and funds needed by each method to accomplish the same survey (section 2.3.3). Moreover, we discuss the potential of GSV as an alternative survey method to CS (sections 2.4.1 and 2.4.2).

2.2 Material and Methods

2.2.1 Target species

Eucalyptus globulus (Tasmanian blue gum) is an evergreen tree native to mainland Tasmania, Bass Strait Islands and southern Victoria (Jordan et al. 1994). It is widely cultivated for paper pulp in Portugal (Potts et al. 2004). Portuguese plantations are usually coppiced every 10-12 years until completing three rotations (Silva & Marchante 2012). This species has a high resprouting capacity (Catry et al. 2013). Dehiscence occurs throughout the year despite being stimulated by fire and hot dry periods (Cremer 1965; Gill 1997; Santos et al. 2015). It has no specialized mechanism of seed dispersal and most of the seed shed occurs in the proximity of the mother-tree (Calviño-Cancela & Rubido-Bará 2013; Cremer 1965, 1977). *Eucalyptus globulus* seedlings / saplings are highly conspicuous due to the bluish color of the juvenile leaves (Johnson 1926; Velikova et al. 2008) and wildlings frequently establish on disturbed sites (Stoneman 1994) such as roadsides bordering plantations (Kirkpatrick 1977; Larcombe et al. 2013). It reaches reproductive maturity at 3-6 years, normally associated with the transition from juvenile to adult foliage (Potts & Gore 1995).

2.2.2 Countrywide car survey

A countrywide car survey was performed in Portugal, aimed at estimating the density of *E. globulus* wildlings established on roadsides adjacent to eucalypt plantations. Roadsides were defined as a strip of public land adjacent to the outer limit of the road, i.e. between the road and the plantation, where cultivation is forbidden. Thus we could assume that eucalypt plants found on these strips were naturally established and not planted. Maintenance of roadsides, mostly mowing operations, is executed at most twice a year in Portugal, especially on the main roads, between April and September, but many other roadsides lack maintenance for longer periods.

Routes for CS were prepared in Geographic Information Systems (GIS) using a road map and a forest map. Routes had to be planned due to the high density of roads and the scattered distribution of *E. globulus* plantations across the country. A total of 38 daily car routes were designed according to the following criteria: cross as many eucalypt stands as possible in one single day; keep a feasible route length; keep eucalypt stands at the observer side avoiding u-turns; avoid roads travelled before; avoid highways because of the minimum speed limit (50 Km h^{-1}); finish each route near accommodation facilities when needed; achieve 3000 sampling areas proportionally distributed among the twelve Portuguese natural regions, according to the percentage of *E. globulus* stands in each region relatively to the total eucalypt area in Portugal (data in Online Resource 1). Portuguese natural regions divide the territory in major geomorphological units, depicting regional differences concerning climatic, geological and edaphic characteristics (Albuquerque 1954). Routes were recorded using a GPS device.

Car surveys were performed between December 2013 and May 2014 by one driver and always the same observer. Sampling units consisted of roadside transects, up to 100 m long and 10 m wide, adjacent to *E. globulus* stands at the reproductive stage, either in first rotation with adult leaves or in the second or above rotations, regardless of tree size and leaf phase. Therefore, we only discarded first rotation stands with eucalypts with juvenile foliage.

The location of the roadside transects was defined spontaneously along each journey, with no car stops, whenever a possible suitable area was detected ahead. Transect limits (start/end) were indicated by the driver using the car odometer. Each transect was surveyed at a constant speed of 30-40km h^{-1}. The speed criterion in CS was adopted in order to keep constant the survey effort in every sampling area, also avoiding car stops for safety reasons. Transects fulfilled the following criteria: presence of an *E. globulus* stand adjacent to the roadside (minimum of 20 m length); roadside with a maximum width of 10 m to assure efficiency in wildling detection; no evidence of recent clearing of vegetation; minimum distance of 1000 m between consecutive transects. In case of partial clearing of vegetation within the roadside width, only the undisturbed strip was considered.

In order to estimate wildling density (plants ha^{-1}), in each transect the observer: should count all wildlings established on the roadside, should estimate transect length (if shorter than 100 m) and should estimate the average roadside width. No other variables were collected along CS. Transect location was recorded through a georeferenced point marked at the end of each transect using a GPS device. A total of 3111 roadside transects was assessed along ca. 15000 km. Given the comparative purpose of this study, all CS transects where GSV survey was not possible were discarded. As a result, in this study we used a sample of 2398 transects (Fig. 1), corresponding to 77% of the initial set of CS transects.

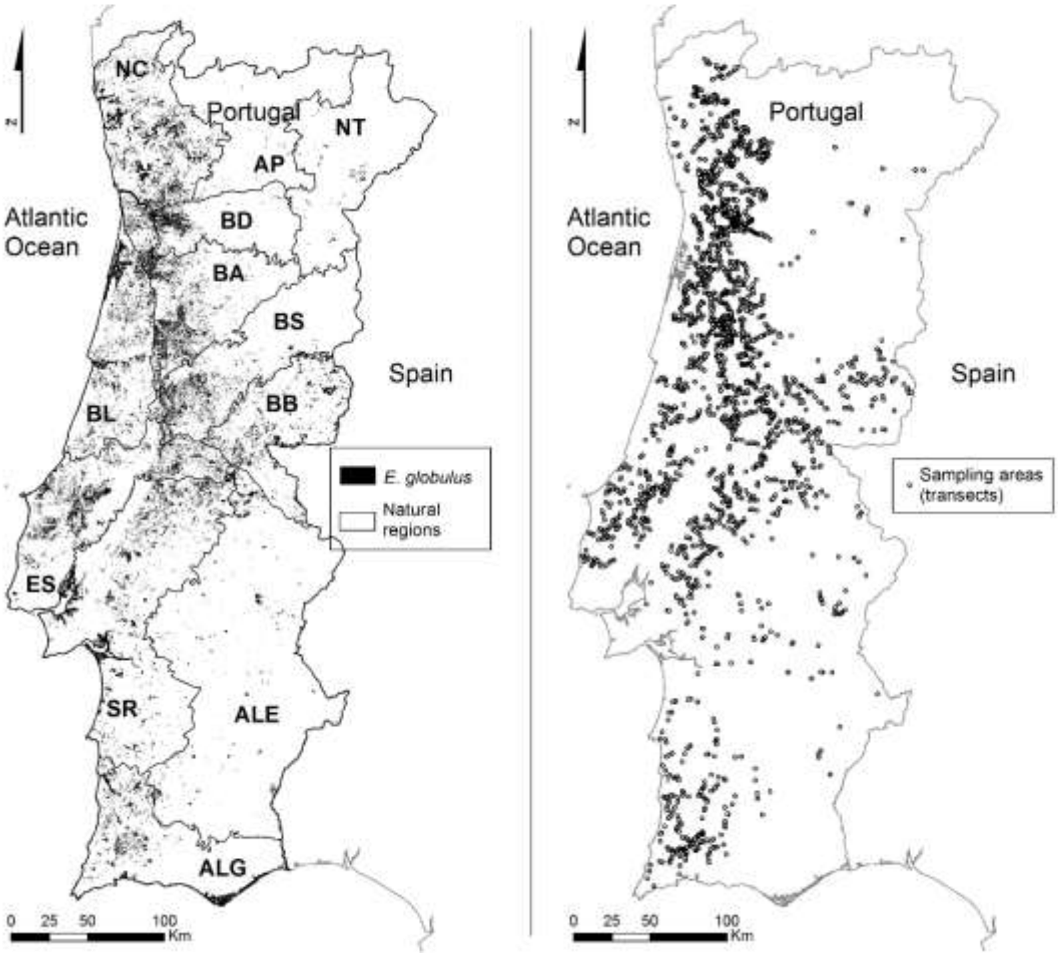

Fig. 1 - Distribution of *E. globulus* stands across the twelve Portuguese natural regions (left) and sampling areas (right) performed by car and GSV. Natural regions: ALE - Alentejo; ALG - Algarve; AP - Alto Portugal; BA - Beira Alta; BB - Beira Baixa; BD - Beira Douro; BL - Beira Litoral; BS - Beira Serra; ES - Estremadura; NC - Noroeste Cismontano; NT - Nordeste Transmontano; SR - Sado e Ribatejo

2.2.3 Countrywide Google Street View survey

Google Street View, specifically the version featured in Google Earth, was used to "revisit" the roadside transects surveyed by car in order to make new estimations of the density of *E. globulus* wildlings. Both GSV survey and CS were performed by the same observer. Georeferenced points gathered along CS, marking the end of each transect, allowed to locate and survey the same extents using GSV imagery. In order to do so, using GIS, road transects 100 m long were drawn from these points backwards along the road, using a road map and the tracks recorded in the GPS device to adopt the right direction. The drawn transects were then converted into a KML file (Keyhole Markup Language) using GIS and imported into Google Earth software, allowing the visualization of each transect, in GSV environment, as a line overlapping the road (check Online Resource 2). Among all transects with available GSV, some transects had to be discarded owing to factors such as recent maintenance and deficient visibility. The observer estimated wildling density similarly to CS and collected some additional local variables (section 2.2.4). GSV imagery included photos taken in the period between January 2009 and October 2010. Time lapse between CS and GSV imagery ranged between 63 and 39 months (mean = 51; SD = 5).

2.2.4 Modeling variables

A total of 16 environmental variables (Online Resource 3), hereafter "regional variables", were gathered from free-access databases for mainland Portugal aiming to produce comparative models of wildling abundance using data from CS and GSV. These were chosen based on works and reports concerning the factors influencing eucalypt establishment (e.g. Águas et al. 2014; Jacobs 1979; Kirkpatrick 1975; Larcombe et al. 2013), and included geographic, edaphic, climatic and wildfire-related variables.

An additional set of seven variables, hereafter "local variables", was collected exclusively along the GSV survey (inexistent in the CS database). These variables were chosen taking into account the existing literature on eucalypt establishment (Águas et al. 2014; Calviño-Cancela & Rubido-Bará 2013; Larcombe et al. 2013; Stoneman 1994), and included stand, site and season-related variables. The "local variables" were used together with the "regional variables" to produce a new model with GSV data.

This set of variables could not be recorded by the CS method since car stops or additional observers would be required. The variable season of survey was not considered in CS since the survey was performed only in winter and spring. In GSV, dates are presented as month-year, so we adopted the classification of meteorological seasons (e.g. winter includes December, January and February) instead of astronomical seasons (Trenberth 1983).

In this paper we will only present the explanatory variables selected as the most important in the modeling exercises. The complete list of variables, with additional information (summary statistics, categories and sources), can be found in Online Resource 3.

2.2.5 Data analysis

In both surveys, wildling abundance was estimated in each transect as the number of plants per hectare, considering the number of detected plants and the estimated roadside length and width. The abundance values were converted into seven categories using an ordinal variable coded as: 0 (no wildlings detected); 1 (>0-100 plants ha^{-1}); 2 (≥100-300 plants ha^{-1}); 3 (≥300-500 plants ha^{-1}); 4 (≥500-700 plants ha^{-1}); 5 (≥700-1500 plants ha^{-1}); 6 (≥1500 plants ha^{-1}). All following statistical analyses were carried out using the R software, version 3.1.1 (R Core Team 2014).

The comparison of wildling abundance categories between GSV and CS (section 2.3.1) was performed using the Spearman's rank correlation and the polychoric correlation (maximum likelihood estimation) (Drasgow 1986), appropriate for ordered categorical variables. The latter was estimated using R package “polychor” (Fox 2010).

In order to test any spatio-temporal bias of the GSV sampling, i.e. the distribution of road transects across the country according to the dates of GSV imagery, we used a qui-square test considering the Portuguese natural regions and the seasons of the GSV imagery (section 2.3.2). With the purpose of complying with the test assumptions, we merged some of the twelve regions taking into account their proximity, resulting in nine regions, and merged the four seasons into two seasons, winter/spring and summer/autumn, starting in the two most stressful seasons in Mediterranean climates (winter and summer) (Orshan 1989).

The following procedures to model wildling abundance were similar to those used in Catry et al. (2015). We used Pearson correlation and the variance inflation factor (vif) (Zuur et al. 2009) to check for correlation and collinearity within the chosen set of explanatory variables. The vif coefficient was estimated using R package ‘AED’ (Zuur et al. 2009). Four variables presented high correlation (all $|r|>0.70$): insolation, evapotranspiration, number of precipitation days and precipitation. From this set, only precipitation was retained for model building. After this initial screening all vif values were lower than 3 and all pairwise correlations were lower than 0.60.

We used BRT models (De'ath 2007) to explore the main drivers of wildling abundance provided by each survey method (CS and GSV). Model building procedures followed Elith et al. (2008) using the ‘gbm’ library (Ridgeway 2009). Models were produced choosing a tree complexity (number of interactions within a single tree) of 5. The learning rate (contribution of each tree to the growing model) was chosen so that each model would be produced with at least 1000 trees (Elith et al. 2008). The optimal number of trees was determined through cross-validation using 10 training sets. Models were built using a bag fraction (proportion of randomly chosen observations) of 0.5. Explanatory variables were ranked according to their relative importance (based on the number of times the variable was selected for splitting, weighted by model improvement and averaged by the number of trees) (Buston & Elith 2011). The influence of each predictor on the response variable was visualized using a partial dependence plot which considered the average effects of the other predictors (obtained using gbm.plot). Spatial autocorrelation in model residuals was taken into account through the Residuals Autocovariate (RAC) approach (Crase et al. 2012) implemented in R package ‘spdep’ (Bivand et al. 2013; Bivand & Piras 2015). The approximate maximum distance between neighbor transects (50 km) was used as the neighborhood radius. Each point within this radius was weighted by the respective squared inverse distance. After estimating the autocovariate, this new variable was included in the set of predictors.

The existence of spatial autocorrelation affecting the response variable was tested using the Moran’s I (Fortin & Dale 2005) implemented in R package ‘ncf ’ (Bjørnstad & Falck 2001). Test significance was obtained through 500 permutations and a sequential Bonferroni correction (Fortin & Dale 2005). The RAC improved the performance of all computed models (CS and GSV data) and ranked as the first or second most important variable at the expense of the relative importance of the other variables, even though the hierarchical importance of the variables was basically maintained.

Three BRT models were created for wildling abundance: one first model for CS data using "regional" variables; one second model for GSV data using "regional" variables"; one third model for GSV data using both "regional" and "local" variables. The purpose of the three models was to compare the first two models in terms of model performance and selected variables using the two survey methodologies (GSV and CS), and to compare the last two models to evaluate changes in model performance and selected variables by adding the ("local") variables collected exclusively using GSV (section 2.3.2).

The explained deviance (relative to the null deviance) and the correlation were used to assess model performance. Standard errors produced during the cross-validation process were used to compute the 95% confidence intervals. Comparison across models was done using the cross-validated proportion of explained deviance and correlation, and assessing the order of importance of explanatory variables. We only retained variables with an importance greater than 2%, in order to limit the comparison to the most influential variables. Detailed model results can be found in Online Resource 4.

Finally, we compared GSV and CS methods in terms of time and funds needed to accomplish the survey (section 2.3.3).

2.3 Results

2.3.1 Wildling abundance comparison

In Fig. 2 we observe some variability of the abundance categories between GSV and CS, except for category 0 (no wildlings), where a large proportion of GSV observations also registered wildling absence (0.61, n=859). The increase in the abundance categories from CS corresponds to a progressive decrease in the proportion of absence observations (category 0) using GSV (from 0 to 6, respectively, 0.61, 0.36, 0.29, 0.19, 0.18, 0.11, 0), and a concurrent increase in the proportion of the higher abundance categories, despite an overall trend for lower wildling abundance registered in GSV (more observations under the x=y line). Even though, for every abundance category from CS, there were still cases of higher abundance estimated using GSV (19.2% of the total transects; n=461). A lower density was estimated using GSV (median [and inter-quartile range] of 0 [0-2] vs. 1 [0-2], paired Wilcoxon signed rank test, V=587697, $p<0.001$). Nevertheless, there was an overall agreement between both indicators (Spearman r = 0.37; polychoric correlation [and standard error] = 0.44 [±0.021]).

2.3.2 Model comparison

The models using CS and GSV data with "regional" variables selected the same explanatory variables with the same hierarchical importance: "precipitation" > "distance to the ocean" > "slope" > "altitude" > "humidity" ≥ "frost". However, GSV model had a lower explained deviance (8.3% vs. 17.9%). The variable "fire" was included only in the GSV model with a marginal importance (Fig. 3).

Overall, we can observe a similar influence of the different variables on wildling abundance. However, some differences are particularly perceptible in a few variables: while in the GSV model there was an increase in wildling abundance towards higher "altitude" and "slope", in the CS model the maximum values were observed in the middle values of both variables (Fig. 3).

The model using GSV data and both "regional" and "local" variables explained 12.4% of the deviance. All "regional" variables lost importance, even though "precipitation" and "distance to the ocean" were kept as the most important variables. Three "local" variables were included: "season" and "roadside slope" as the third and fourth more important, and "coppicing" with a lower importance (Fig. 4).

A qui-square test showed that "season", i.e. the season corresponding to the date of the GSV imagery used to survey each transect, was not independent of the region (p-value < 0.001), with no particular pattern between the type of season (winter/spring or summer/autumn) and the type of region (higher or lower wildling abundance).

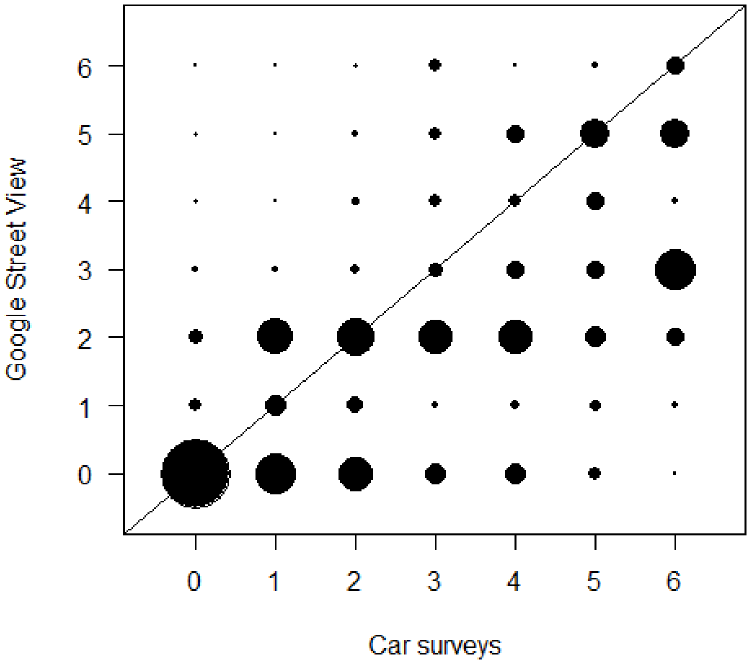

Fig. 2 - Plot of the different combinations of frequencies of the ordered categories of wildling abundance as measured by GSV versus CS. Frequencies (proportional to symbol size) are expressed as proportions of the total number of transects for each car survey abundance level (that sum to 100%). For example, for the transects classified as abundance level 0 in car surveys (n=1131), the proportions classified as 0 to 6 in GSV were, respectively, 0.61, 0.11, 0.13, 0.05, 0.04, 0.04, and 0.02. The x=y line is also drawn for comparison. If abundance measures in GSV and CS were exactly the same all symbols would be concentrated in the diagonal

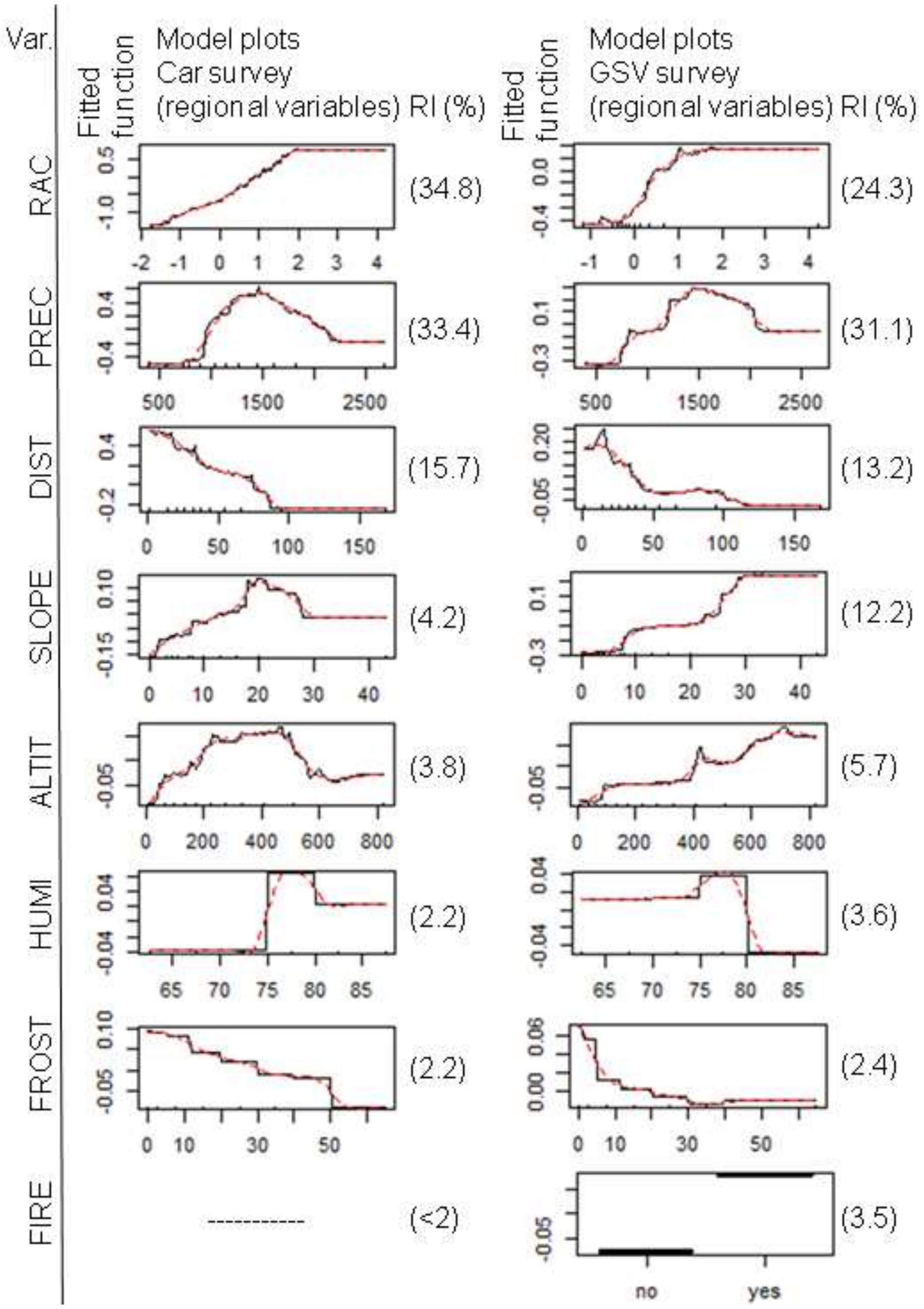

Fig. 3 - Partial dependence plots and relative importance (RI) of the variables (Var.) influencing wildling abundance in Portugal, from the CS and GSV models with regional variables (models with RAC). A smoothed version of the fitted function is also shown (dashed line). Deciles distribution of observations is represented in the horizontal axis through tick marks. RAC - Residual Autocovariate; PREC - mean annual precipitation (mm); DIST - distance to the ocean (Km); SLOPE - general slope (º); ALT - altitude (m); FROST - mean annual number of frost days (days); HUMI - mean annual relative humidity (%); FIRE - burnt in the last five years

2.3.3 Cost-time effectiveness

In order to accomplish 2398 transects, the CS approach took 29 working days (i.e. 29 daily car routes). It was accomplished by two persons (58 person-days), one driver and one observer. Besides wages, the costs included travelling, on a total of 11692 km (0.36€ per km, according to the official rate for Portuguese public institutions), accommodation and daily allowances. Total budget for CS was 7229.8€. These values were computed for the 2398 transects surveyed using both methods. The GSV approach required one person and 18 working days at the office. Wages were the only expense. Total budget for GSV was 674.2€. Time accounted for each of the two methods does not include other tasks than the survey itself (e.g. planning routes). Also, none of the budgets included equipment costs (investment and maintenance).

2.4 Discussion

2.4.1 Did both methods yield the same results?

Despite an overall agreement between methods in the estimation of plant abundance, we found virtually all combinations of abundance categories across methods and an overall trend for lower wildling abundance estimated using GSV (Fig. 2).

Regardless of the survey method, we would expect some variability on wildling abundance due to the time lapse of 39-63 months between the two surveys (GSV imagery and CS). In fact, within such time lapse, stressful climatic events such as frost (Almeida et al. 1994) or droughts (Humara et al. 2002) could result in wildling mortality, or particular climatic conditions could favor plant survival and growth (Calviño-Cancela & Rubido-Bará 2013; Orshan 1989). Similarly, roadside maintenance could contribute either towards an increase or a decrease in wildling abundance, by providing favorable conditions for establishment through the reduction of plant competition (Kirkpatrick 1977; Stoneman 1994), affecting resource availability (Davis et al. 2000) or mowing young wildling cohorts that did not survive and resprout (Catry et al. 2013). Therefore, the number of present wildlings could maintain or evolve in both directions (increase or decrease) for each of the 2398 transects.

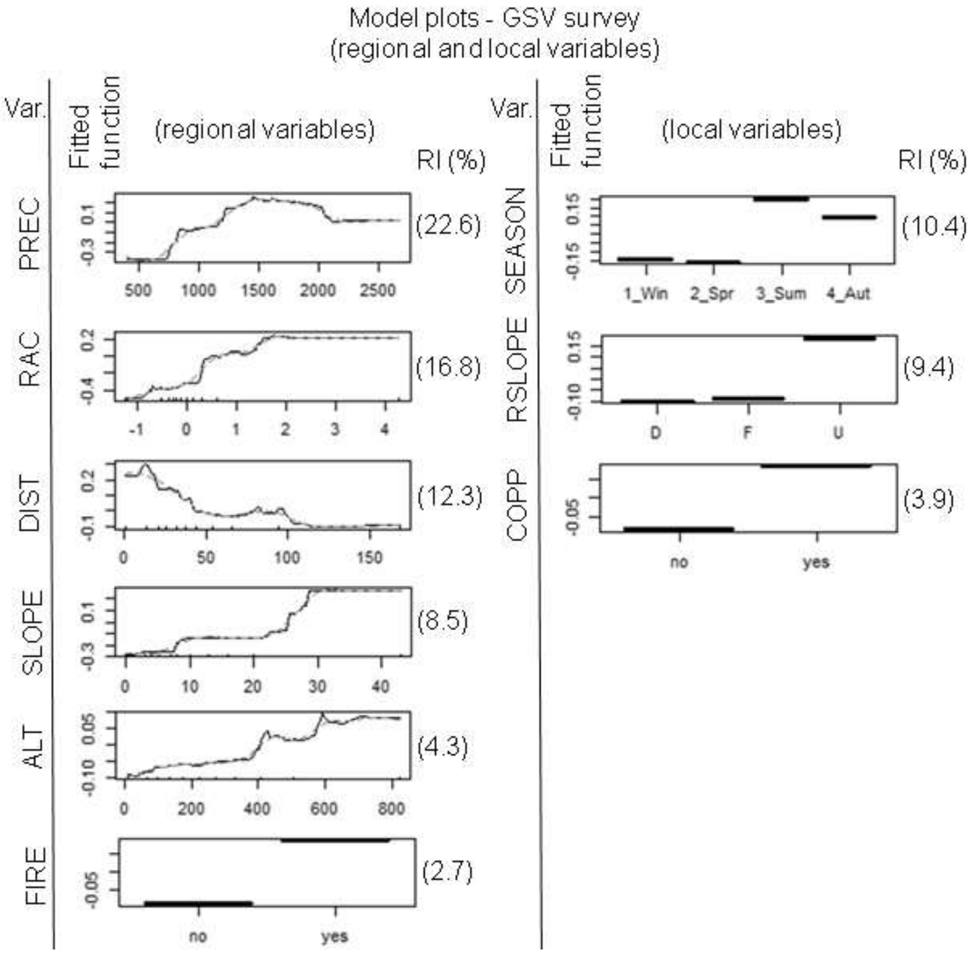

Fig. 4 - Partial dependence plots and relative importance (RI) of the variables (Var.) influencing wildling abundance in Portugal, from the GSV model with regional and local variables (model with RAC). A smoothed version of the fitted function is also shown (dashed line). Deciles distribution of observations is represented in the horizontal axis through tick marks. PREC - mean annual precipitation (mm); RAC - Residual Autocovariate; DIST - distance to the ocean (Km); SLOPE - general slope (º); ALT - altitude (m); FIRE - burnt in the last five years; SEASON - season of survey; RSLOPE - roadside slope; COPP - coppiced stand

However, we believe that, regardless of the time lapse, underestimation of plant abundance is likely to occur using GSV. Similarly to other studies (Mazerolle & Blaney 2010; Olea & Mateo-Tomás 2013), we experienced difficulties related to GSV imagery sharpness, contrast and level of ambient light, the latter related to occasional shadowing, sun height and cloudy sky. As a result, many plants probably went unnoticed, especially when smaller, located within dense green vegetation, in shadowed areas or more distant from the road.

Although the selected variables were basically the same, the performance of the GSV model was much lower than in the CS model, probably owing to a lower plant detection efficiency of the former. In this study, for comparison purposes, GSV surveys were limited to the areas surveyed by car, but one can assume that an increase in sample size, easily achievable using GSV in opposition to CS, most likely would increase model performance.

Even so, both methods highlighted the same explanatory variables for wildling abundance, ordered with the same hierarchical importance and with similar influence on wildling abundance (Fig. 3). There were however small differences between models that, together with some of the additional "local" variables, may enlighten about method specificities and thus are worthy of discussion.

In the CS, greater wildling abundance was found in areas with intermediate slope and altitude, whereas in the GSV survey it was registered in steeper slopes and more elevated areas (Fig. 3). In these latter areas we frequently find sharp road cuts displayed very near the road, situations where it is harder to detect plants in a moving car when compared to static imagery with close-up photos, which may partly explain the enhanced plant detection in these cases using GSV. The same logic may also help explaining why greater wildling abundance was found in upslope roadsides using GSV in relation to flat and downslope roadsides (Fig. 4). Also, the height of the GSV cameras on top of vehicles (Hoelzl & Marie 2014) may have favoured the detection of plants established overhead in wide steep roadsides (see an example in Online Resource 2).

We also hypothesize that the lower wildling abundance along winter/spring "seasons", depicted in the GSV model using both "regional" and "local variables" (Fig. 4), may be partly related with plant conspicuity under different conditions of vegetation and light. First, using GSV imagery, it may be particularly harder to distinguish and identify plants within a densely green-vegetated background, normally found during wet seasons, in our case occurring in winter/spring. Second, GSV imagery is more likely to lack better light conditions in wet seasons due to shadowing effects, either by land obstacles or clouds, worsened by the lower height of the sun arc in the sky in the winter at north hemisphere mid-latitudes, meaning less solar radiation and larger shadowed areas. However, we cannot discard environmental processes concurrently influencing plant abundance and detectability, such as an enhanced plant growth in spring, when neither extreme temperatures nor water deficits constrain plant development, resulting in more detectable plants in the following summer. Afterwards, wildling abundance suffers a progressive reduction caused by summer drought-induced mortality and later by winter cold-induced mortality (Orshan 1989), while plant detectability also decreases as mentioned above. These assumptions could not be strengthened using CS data since it was performed only in the winter/spring. The existence of a heterogeneous distribution of survey seasons across regions may be a relevant issue for some studies. However in our case the interactions between spatially-dependent variables and season were automatically modelled by the BRT approach (Elith et al. 2008).

Models also differed at the inclusion of recent wildfires as a driver of wildling abundance only in the GSV model, influencing it positively in accordance with several studies (Águas et al. 2014; Larcombe et al. 2013; Santos et al. 2015). Note however that the two survey periods were different. As a result, there was a greater number of surveyed areas in recently burnt areas in the GSV survey (240 vs. 90), most probably explaining why this variable was not selected in the CS model.

Coppicing, a "local" variable selected in the GSV model, also showed a positive influence on wildling abundance, even though in this case it probably has an ecological explanation. Tree felling may promote a massive propagule release and, in particular cases, surface disturbance on roadsides due to logging operations may provide more suitable conditions for wildling establishment (Águas et al. 2014).

In conclusion, despite some local variability in plant abundance, the results from both survey methods depicted the same environmental drivers of plant abundance, which is key information for invasion ecology and to formulate effective measures for plant control and monitoring (Pyšek et al. 2009; Richardson & Rejmánek 2011; van Wilgen et al. 2011; Vicente et al. 2010).

2.4.2 Is Google Street View a viable alternative to car surveys?

We may argue that different studies can be conducted using GSV as an alternative survey method to CS, particularly in plant and invasion ecology, based on three assumptions: a) the existence of a dense GSV network in several world regions (check the network at Google 2015); the conspicuity of many invasive plants; c) the importance of roadsides for the establishment and spread of invasive plants (Christen & Matlack 2006; Forman & Alexander 1998). However, the use of this technology and its efficiency depend on a variety of factors.

On the other hand, there are important advantages in this method when compared to CS. Both perspectives are worthy of discussion in this section.

Our results suggest that an intensive large-scale survey is faster and more economical using GSV than CS. In this study, considering a similar sampling effort, we estimated that the GSV survey required ca. 62% of the time (18 vs. 29 working days) and 9% of the funds (674.2€ vs. 7229.8€) needed to accomplish the CS. In a study of vulture nesting habitats on cliffs, Olea and Mateo-Tomás (2013) concluded that a survey using GSV would take ca. 64% of the time and 50% of the funds needed in a field-based survey, despite not taking into account some travelling costs. In our case, given the conditions presented in section 2.2.2, planning of the CS routes required intense labor (nearly one day of planning for one daily journey), which was not accounted for the comparison of the total time spent by each method, since the GSV survey benefited from this preparatory work. Still, locating sampling areas for an autonomous GSV survey would be faster using automatic processes in GIS. For example, creating a regular grid of points over the study area and automatically displacing them into the nearest road.

The main determining factor for the time gain using GSV was the swift transition between sampling areas using the KML file in Google Earth, while in CS considerable time was spent on travelling before, during and after the survey. However, similarly to Mazerolle and Blaney (2010), the assessment of each transect individually was nearly twenty times slower using GSV (average of 216 seconds vs. 9-12 seconds at 30-40km h^{-1}), even though a greater amount of information was collected using GSV.

The lack of time restrictions is one of the main advantages of GSV when compared to CS, since one can perform the survey anywhere, anytime and as many times as needed. It can be particularly useful to revisit areas to confirm or collect additional information. In our case, it allowed us to gather important information that improved model performance by adding new variables (Fig. 4), which in turn provided insight both into method limitations and species ecology (section 2.4.1). The collection of these variables along CS would require car stops or additional observers, therefore involving additional resources.

The primary factors constraining the use of GSV in alternative to CS are the existence, distribution and density of the GSV network throughout the study area, and the first difficulty one will presently find is the inexistence of an open-access vectorial map featuring the GSV coverage. Such a map would facilitate sampling design and make it more efficient, especially if combined with the available imagery dates for each road, allowing the opportunity to check the bias of the GSV road coverage, either in space (environmental gradients) or in time (seasons). The current alternative is an exploratory survey aiming to depict the spatial and temporal availability of the GSV network.

In this study, the GSV method apparently failed to detect more plants than the CS method. In the case of *E. globulus*, the detection of wildlings through photographs may be more difficult since wildlings normally appear as small individuals with a sparse distribution. Smaller plants forming small patches are likely harder to detect using GSV imagery (Mazerolle & Blaney 2010), meaning that a GSV survey may be less efficient than a CS at detecting some invasive plants in the early stages of invasion.

We may assume that most of the plant species eligible for CS may be also eligible for GSV survey, but plant conspicuity must be more important for the efficiency of the GSV method (Mazerolle & Blaney 2010). However, in many cases, conspicuity may vary according to seasonal processes such as flowering (e.g. Shuster et al. 2005; Wilcox 1989) and leaf presence in deciduous plants (e.g. McAvoy et al. 2012), while many plants may become practically undetectable in certain seasons like snow season, thus the date of the GSV imagery may be a constraint in some cases. Moreover, we also found that the date of GSV imagery may be seasonally biased across regions, i.e. different regions may have a predominance of certain months and seasons. Unlike CS, we cannot choose the survey period using GSV (Mazerolle & Blaney 2010; Olea & Mateo-Tomás 2016; Rousselet et al. 2013). Finding suitable periods for a given species' phenomorphology can be challenging in some cases. Nevertheless, we found imagery practically from all seasons in all regions, indicating that it may be possible to search for seasonal phenomena over large extents using GSV.

Despite some limitations of this technology as a survey method, we believe GSV can be used autonomously for plant detection in different world regions targeting different plant species, as long as sufficiently conspicuous and normally located in roadsides or nearby areas. Throughout the GSV survey, we repeatedly found other suitable plant species, considered invasive also in other world regions (Pyšek et al. 2009; Richardson & Rejmánek 2011) such as *Acacia dealbata* Link (Lorenzo et al. 2010), *Ailanthus altissima* (Mill.) Swingle (Sladonja et al. 2015) and *Cortaderia selloana* (Schult. & Schult.f.) Asch. & Graebn (Doménech et al. 2005).

Alternatively, GSV can be used complementarily to CS. Catry et al. (2015) "revisited" with GSV areas surveyed by car and combined the results from both methods in order to overcome temporal factors (e.g. roadside maintenance) that may lead to an underestimation of plant abundance. Also, CS may be used where GSV is not available, even though it is recommended to avoid biasing regions according to survey methods since the accuracy between methods may differ.

The GSV method may respond to the need for a more cost-effective survey method (e.g. Kalkhan & Stohlgren 2000; Shuster et al. 2005; Stohlgren et al. 1998) to fill the gap of knowledge on the large-scale distribution of some invasive species in many regions (Pyšek et al. 2009). Considering the cost-efficiency of GSV, we may say that this tool is particularly appealing and useful in a time when the efficiency of resource allocation is essential (Buhlea et al. 2005; Norton & Warburton 2015; Wilson et al. 2007) and many researchers deal with limited budgets or experience funding cuts.

2.5 Conclusion

In this work, we were able to replicate an exhaustive countrywide survey conducted by car, using GSV as a remote sensing tool, to assess the abundance of plants established on roadsides. We compared the results from both methods and concluded that there was an overall agreement in the estimation of plant abundance, despite a tendency for fewer plants being detected using GSV, probably explained by a lower plant conspicuity in many locations due to image sharpness, contrast and level of ambient light. The plant abundance models produced by both survey methods were very similar in terms of selected explanatory variables and its influence in wildling abundance, even though the GSV model had a lower explained deviance, probably related to the lower efficiency in plant detection. The use of GSV also allowed the collection of additional variables that improved model performance and provided new insight into species ecology (positive influence of coppicing on *E. globulus* wildling abundance) and possible limitations of the survey methods, such as a variable efficiency of GSV in plant detection across seasons and the possibility of a spatio-temporal bias of the GSV network. We conclude that GSV may be a cost-efficient alternative method to CS and we forecast this will be a widespread tool among researchers dedicated to road ecology, particularly in large-scale vegetation assessments.

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Chapter 3: Investigating the invasiveness of *Eucalyptus globulus* in Portugal: site-scale drivers, reproductive capacity and dispersal potential

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3.1 Introduction

Global species distributions are a consequence of diversification, dispersal and the rearrangement of land and sea over the geological time. Most taxa today are confined to geographic regions that are delineated by natural barriers to dispersal (Darwin 1859; Elton 1958). However, movement by humans has allowed many species to overcome such barriers, either through premeditated or inadvertent human will (Hulme 2009; Meyerson & Mooney 2007). Some of these exotic species have been a matter of great concern because of their ability to spread, causing negative ecological and economic impacts (Pimentel et al. 2001; Vilà et al. 2011).

Eucalyptus L'Hér. (Myrtaceae) is a large, diverse plant genus, comprising over 800 species, largely native to Australia (Nicolle 2018). Since the end of the 18th century several eucalypt species were introduced to different regions of the world (Potts 2004; Silva-Pando & Pino-Pérez 2016). Some eucalypt species were well adapted to the exotic environments and became widely exploited for timber, firewood, windbreaks, soil retention and, more recently, paper pulp production (Jacobs 1979; Potts 2004). Currently, it is estimated that eucalypt plantations cover around 20 million ha outside the native range (Rejmánek & Richardson 2011).

There has been a growing concern about the invasive potential of some eucalypt species. Several eucalypt species are considered to be naturalised (Pyšek et al. 2017) and a few species are regarded as invasive in different regions of the world (Rejmánek & Richardson 2013). Naturalised plant species have been defined as species able to generate reproductive offspring, normally near the parents, to form self-perpetuating populations, while invasive plant species are able to originate offspring distant from the parents, frequently causing negative ecological and economic impacts (Blackburn et al. 2011; Richardson et al. 2000).

A prior literature review, developed in the frame of another research initiative, revealed around 30 publications (papers and theses), assessing aspects of eucalypt invasiveness using wildling surveys or sowing experiments, mostly published in the last decade. These studies investigated the invasiveness of dozens of eucalypt species in different countries, such as Brazil (e.g. Miolaro et al. 2017), South Africa (e.g. Forsyth et al. 2004), USA (e.g.

Callaham Jr et al. 2013), Spain (e.g. Calviño-Cancela et al. 2018), Portugal (e.g. Fernandes et al. 2016) and Australia (e.g. Calviño-Cancela & van Etten 2018). *Eucalyptus globulus* Labill. is one of the most extensively cultivated eucalypt species (Rejmánek & Richardson 2011), and is probably the most widely studied eucalypt species in terms of invasiveness. Also, it is considered to be the most widely occurring invasive eucalypt species (in seven out of 15 geographic regions of the world; see Rejmánek & Richardson 2013).

Eucalyptus globulus is native to SE Australia, including the Bass Strait Islands and Tasmania (Jordan et al. 1994). It was introduced throughout the world in the 19th century and plantations expanded rapidly in several countries in the late 20th century, especially to feed the paper and pulp markets (Potts et al. 2004). Portugal grows more *E. globulus* than any other country (by area), covering more than 800,000 ha, around 9% of the country (Deus et al. 2018a; ICNF 2013). The species has a wide environmental tolerance and is able to naturally establish in different regions of Portugal (Catry et al. 2015).

Studies assessing factors associated with *E. globulus* wildling establishment have shown that climate (Catry et al. 2015; Fernandes et al. 2017; Fernandes et al. 2018; Larcombe et al. 2013), plantation-related variables such as plantation size and age (Águas et al. 2017; Fernandes et al. 2018), fire (Calviño-Cancela et al. 2018; Larcombe et al. 2013), post-fire management (Águas et al. 2014), soil cover and disturbances (Fernandes et al. 2018; Kirkpatrick 1977), and the invaded land cover type (Calviño-Cancela & Rubido-Bará 2013; Fernandes et al. 2016; Fernandes et al. 2018) can all be important. Despite previous work investigating *E. globulus* invasiveness, knowledge gaps persist.

Little is known about the demographics of *E. globulus* wildling populations. Understanding the developmental stage of wildlings (e.g. size and transition to adult foliage) provides information about demography such as the number of cohorts and their survival rates, and is also crucial for making conclusions about effective recruitment because small *E. globulus* wildlings experience high levels of mortality (Calviño-Cancela & Rubido-Bará 2013; Fernandes et al. 2017). Few studies assess the height of naturally established wildlings (e.g. Águas et al. 2014; Fernandes et al. 2018) or the transition to adult foliage (e.g. Calviño-Cancela & van Etten 2018). Moreover, to our knowledge, no study investigated the occurrence of wildlings bearing reproductive organs. The onset of sexual maturity is of particular importance, because it is key for determining *naturalisation* and *invasion* (Richardson et al. 2000), and has not been studied in exotic *E. globulus*.

Few studies investigated the natural establishment of *E. globulus* wildlings along the edges of plantations. The edges of plantations are of particular importance because they represent the point of potential escape for wildings from cultivation, and observations on *E. globulus* plantations in Australia show that flowering seems to be more abundant in the trees located along the plantation edges (Barbour et al. 2008), meaning that propagule pressure is probably higher at these locations. Moreover, forest edges form transition zones between two adjacent ecosystems, featuring particular biotic (e.g. species diversity) and abiotic (e.g. microclimate) conditions (Murcia 1995). However, among the few studies investigating wildling establishment along plantation edges, the role of site-scale factors such as soil cover, local topography or slope aspect, was either not investigated (Águas et al. 2017) or inconclusive (Larcombe et al. 2013). It is known that fine-scale factors such as soil moisture, vegetation cover and slope are important in native eucalypt recruitment in Australia (Bailey et al. 2012; Wilson & Gibbons 2014). A recent study conducted in Portugal, using survey plots outside *E. globulus* plantations, also emphasised the importance of site-scale factors such as soil cover and soil disturbance in *E. globulus* recruitment (Fernandes et al. 2018).

Seeds of *E. globulus* do not have any long-distance dispersal adaptation, being normally dispersed by gravity and wind (Booth 2017; Cremer 1977). Nevertheless, seeds of *E. globulus* have been recorded 80 m away from plantations in NW Spain (Calviño-Cancela & Rubido-Bará 2013). Previous studies assessing the dispersal of wildlings from plantations (Calviño-Cancela & Rubido-Bará 2013; Fernandes et al. 2016; Larcombe et al. 2013) have not considered the importance of the primary vectors of dispersal, wind and water. Wind is probably the primary dispersal vector of eucalypt seeds (Calviño-Cancela & Rubido-Bará 2013; Cremer 1977), and the orientation of the plantation in relation to the prevailing wind may be an important factor determining the dispersal distances (McBride et al. 1988). Water has also been implicated in the dispersal of eucalypt seeds (Forsyth et al. 2004; Kirkpatrick 1977), and Larcombe et al. (2013) observed *E. globulus* wildlings established 175m from a plantation down slope in a drainage line. Therefore, the presence of drainage lines (and presumably ephemeral running water) may be associated with increased wildling dispersal distances.

The goal of this study is to contribute to the understanding of *E. globulus* invasiveness, by addressing some important, but poorly explored topics. We surveyed wilding establishment along the edges of *E. globulus* plantations, and assessed dispersal into adjacent areas in Central Portugal. In particular, this study aimed to: a) assess the local site-scale factors influencing presence and abundance of *E. globulus* wildlings; b) quantify the size, foliage development (adult/juvenile), and proportion of reproductive wildlings; c) investigate the effective dispersal distance of wildlings from plantations, including the distribution of developmental classes; and d) assess factors affecting dispersal distance.

3.2 Material and Methods

3.2.1 Study region

The study was conducted in Central Portugal (Fig. 1). Climate is typically Mediterranean, with hot, dry summers and moist winters. Mean annual precipitation is 652 mm, registering minimum values in July (4 mm) and maximum values in November (107 mm). Mean annual temperature is 17 ºC, ranging from 10 ºC in January to 24 ºC in August (climatic data retrieved from IPMA 2018). The topography is irregular, featuring predominantly rugged terrain of hills and valleys, and river plains to a lesser extent. Sampling units, composed of survey plots and survey transects associated to *E. globulus* plantations, were located within latitudes 39°32'02'' N and 38°47'04'' N (distributed within a latitudinal distance of 83 km), and longitudes 7°46'52'' W and 9°00'08'' W (longitudinal distance of 106 km). Altitude of the sampling units ranged between 30 and 307 m. Plantations of *E. globulus* are widespread in this region, occupying c. 11% (c. 92,000 ha) of the area delimited by the referred coordinates.

3.2.2 Field surveys

3.2.2.1 Survey along plantation edges

We performed a survey along the edges of *E. globulus* plantations aiming to assess wildling development and the influence of site-scale factors on the establishment of *E. globulus* wildlings. All *E. globulus* plantations were owned by a Portuguese pulp company (Altri Florestal S.A.). The age of plantations ranged between 4 and 42 years (mean=23.4; SD=8.1). Since *E. globulus* reaches sexual maturity in plantations at 3-4 years of age (Potts & Gore 1995; Potts et al. 2008), plantations were composed of potentially reproductive individuals. The maximum stem age of the surveyed plantation was 12 years-old, meaning that flowering could have started 8-9 years before. In Portugal, plantations are typically managed by coppicing every 10-12 years for 2-3 rotations.

The survey design was similar to a study performed in Australia by Larcombe et al. (2013). The edge (perimeter) of a plantation was defined as a virtual line crossing the boles of the outer trees. The perimeter of the plantations was divided in different edges according to the edge aspect. In this way, a single plantation could comprise distinct edges, which were assessed separately using different transects. In each transect, the survey was performed on 10x10 m plots disposed along the plantation edge. In this way, the perimeter of the plantation divided the plot into two halves, i.e. two adjacent 5x10 m subplots, one inside (*inside subplot*) and one outside (*outside subplot*) the plantation. We aimed to accomplish a paired-plot survey within each transect, featuring a plot where wildlings were present (*presence plot*) combined with a plot where wildlings were absent (*absence plot*).

A *presence plot* was established in the first opportunity to frame a minimum of five wildlings within the plot area, regardless of their size and location (*inside* and/or *outside subplots*). The corresponding *absence plot* was established in the first available wildling-free plot area in either direction along the plantation edge, at a minimum distance of 50 m from the *presence plot* and 15 m away from any other wildling. Minimum distance between *presence plots* was 200 m. Due to the recurrent presence of *E. globulus* wildlings, it was not possible to couple an *absence plot* to every *presence plot*. Therefore, the survey included more *presence* than *absence plots*. We discarded all plantation edges distanced less than 50 m from another *E. globulus* plantation to reduce the chances of registering wildlings originating from other plantations. In the *outside subplot*, we subtracted the area of any surface unsuitable for wildling establishment (e.g. road).

In the *presence plots*, for each subplot (*inside* and *outside*), *E. globulus* wildlings were counted and categorised according to four size classes: ≤0.5 m (*size 1*); 0.5-1.3 m (*size 2*); 1.3-3.0 m (*size 3*); >3 m (*size 4*). For each size class, we also registered the number of *reproductive wildlings*, i.e. wildlings bearing reproductive organs such as flowers or fruits. Wildlings were distinguished from planted individuals by their irregular location and size, within the regularly spaced and similarly sized planted *E. globulus* trees. Wildlings were also distinguished from resprouts by the presence of a lignotuber. *Eucalyptus globulus* is a proficient resprouter, able to regenerate through epicormic strands and from basal buds (Catry et al. 2013).

In each survey plot, including *presence* and *absence plots*, we collected the following data: a) *number of capsules* on each of the ten planted eucalypts closest to the plot centre (visual estimate using binoculars), within a maximum distance of 25 m (categorised as 0: no capsules; 1: between 1-10 capsules; 2: 11-100 capsules; 3: 101-1000 capsules; 4: >1000 capsules); b) *edge aspect*, i.e. direction of exposure of the plantation edge (four cardinal directions); c) *plot topography*, i.e. topographic format of the plot surface (flat; valley; slope; ridge); d) *plot aspect*, direction of terrain inclination (flat; four cardinal directions, later reclassified north and west to *wet*, and south and east to *dry*,); e) *plot slope* (degrees of inclination); and f) the percentage of the plot covered by four soil cover types: bare ground, litter, herbs and shrubs.

A total of 103 edge transects were performed in 67 *E. globulus* plantations, including 129 *presence plots* and 53 *absence plots*. Field work was conducted between January and May 2014.

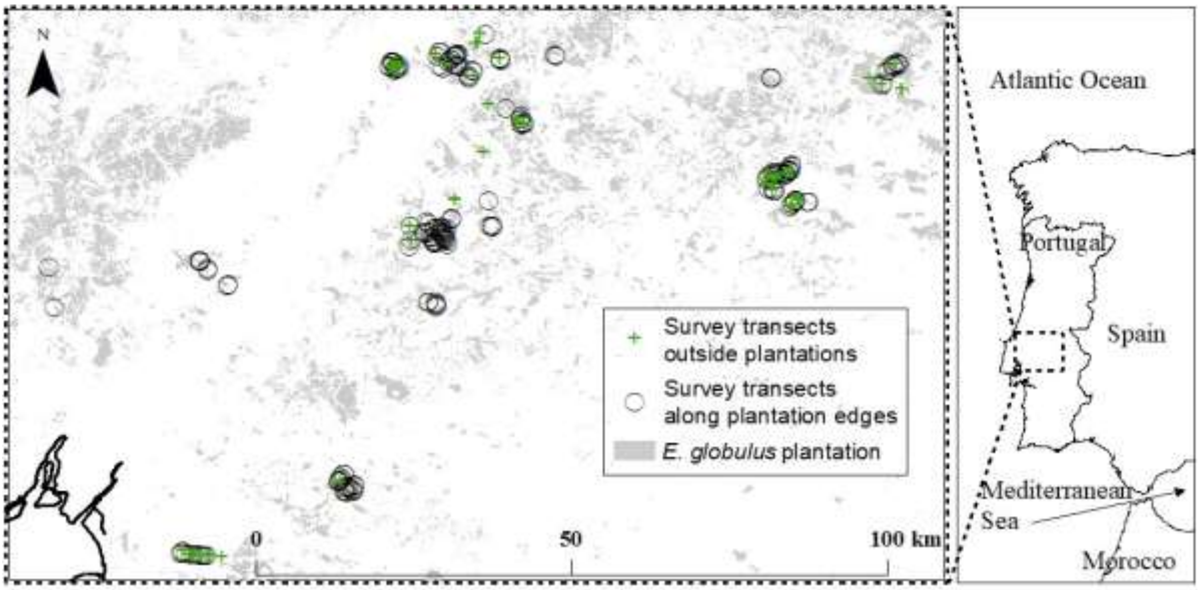

Fig. 1 - Distribution of *Eucalyptus globulus* plantations and sampling areas in the study region

3.2.2.2 Survey outside plantations

We surveyed *E. globulus* wildlings established outside plantations using transects perpendicular to the plantation edges. This survey aimed to record the distance of wildling dispersal from the plantation together with wildling development. The establishment of a perpendicular transect was triggered by the detection of one *E. globulus* wildling at a distance greater than 10 m from the plantation edge. Transects had a maximum length of 80 m, corresponding to the maximum seed dispersal distance registered in *E. globulus* plantations in NW Spain (Calviño-Cancela & Rubido-Bará 2013). Minimum distance between transects was 100 m to avoid registering the same wildlings twice. Transects were established in areas with no other *E. globulus* plantations nearby, to avoid recording wildlings from other plantations.

The survey was conducted by one observer walking along the transect. Each step, previously calibrated, corresponded to 1 m length. At each step, the researcher looked at both sides and recorded the number and developmental stage of wildlings at that specific distance. Since the observer should not move away from the transect in order to have the same detection criteria across transects, we decided not to collect any specific traits which would require closer look, such as plant height and the existence of reproductive organs. In alternative, we characterised the developmental stage of wildlings using the conspicuous juvenile/adult foliage of *E. globulus*. *Eucalyptus globulus* is strongly heteroblastic, featuring strikingly different juvenile and adult foliage, making this developmental transition conspicuous and easily detectable (Johnson 1926; Velikova et al. 2008). We used the conservative assumption that wildlings with juvenile foliage are non-reproductive because the onset of sexual reproduction does not normally occur until after the transition to adult leaves (Potts et al. 2008), although juvenile wildlings with reproductive structures were identified in this study (see results; Fig. 2). For each transect, we collected the following data: *transect land cover*, i.e. predominant land cover along the transect (agriculture; shrubland; pine plantation; riparian zone; *montado*; paddock); *drainage line presence*, i.e. occurrence of a natural drainage line running away from the plantation edge (yes; no). *Montado* is a traditional agroforestry system in Portugal characterised by an open forest of native oaks. A total of 49 survey transects were performed outside 33 *E. globulus* plantations (Fig. 1). Field work was conducted between January and May 2014.

3.2.3 Data analysis

3.2.3.1 Wildling presence

This analysis aimed to investigate the site-scale factors (see Section 3.2.2.1) influencing the *presence* of *E. globulus* wildlings, regardless of their size, along the edge of plantations. We used wildling presence-absence in the 10x10 m survey plots as a binary response variable. This analysis contrasts local conditions that are very adequate (at least five plants in 100 m^2) for wildling establishment (recruitment and persistence in *presence* plots) with local conditions that are inadequate for establishment (*absence* plots). *Presence* was preferred to *density* as a dependent variable, because of poor performance of the *density* models that were produced at an exploratory stage of the analysis. Explanatory variables included the site-scale variables collected in each subplot (see Section 3.2.2.1) and the *stem age* of the planted *E. globulus* trees, i.e. the time (years) since planting or coppicing, information that was provided by the plantation owner. The variable *stem age* was included as a covariate to remove predictable variation on some site-scale factors associated with tree development. For instance, older stems should correspond to lower soil exposure and higher reproductive potential. Also, this factor (*stem age*) is constant among survey plots located within the same plantation. The reproductive capacity of the plantation was determined using the *maximum capsule score* from the 10 trees associated with the plot.

We used logistic regression to assess the influence of site-scale factors on the presence of *E. globulus* wildlings. Due to the hierarchical nature of our sampling design (*transects* nested within *plantations*), we used generalised linear mixed models (GLMM) with a binomial distribution and logit link function, using the R package *lme4* (Bates et al. 2017). We fitted a model using *presence-absence* as the dependent variable, with *transects* nested within *plantation* as random effects. In order to avoid multi-collinearity between explanatory variables we computed variance inflation factors (VIF) to select the final set of potential predictors with VIF < 4 (Zuur et al. 2009) using the R package *usdm* (Naimi 2017).

The following fixed effects were included in this order: *stem age*, *bare ground cover*, *litter cover*, *shrub cover*, *herb cover*, *plot topography*, *plot slope*, *maximum capsule score*, *plot aspect* (*wet* and *dry*) and *edge aspect*. We used a backwards step model simplification process where non-significant terms were removed from the model, starting with categorical terms with the highest *P*-value. The reduced model was compared to the previous model using the likelihood ratio test. If the reduced model was significantly worse than the previous model, the dropped term was reinstated. Alternatively, the reduced model was accepted. This process was repeated until no non-significant terms remained. Residual diagnostics were conducted on simulated scaled residual plots, produced using the R package *DHARMa* (Hartig 2017). This procedure produces interpretable residuals from hierarchical mixed models. Model fit was assessed by conditional R^2 (Nakagawa & Schielzeth 2013), using the R package *piecewiseSEM* (Lefcheck 2016).

3.2.3.2 Wildling abundance

We used a paired Wilcoxon test to compare the density (number of wildlings per area) of each wildling size class between the *inside* and *outside subplots*, retaining exclusively the *presence plots* (plots featuring a minimum of five wildlings).

We explored the influence of site-scale factors on the abundance of young plants, as an indicator of site suitability for recruitment. As a response variable, we used wildling density (number of wildlings per area). We restricted this analysis to the abundance of smaller wildlings (*size 1*; <50 cm) in the *inside subplots* of the *presence plots*. This criterion was used for two reasons: first, younger wildlings should reflect more accurately the site-scale conditions registered at the date of the survey; second, *inside subplots* are less prone to disturbances (e.g. vehicles; people). Unlike the previous analysis, we discarded all *absence plots* in order to retain only locations where wildlings find conditions to establish. We used the same set of explanatory variables as in the presence-absence analysis, including *stem age* and site-scale variables (see Section 3.2.2.1).

Since density data were highly skewed and could not be transformed to meet the assumptions of generalised models, we used regression trees as an alternative approach. We chose multivariate regression trees (MRT) to assess wildling density. Among other features, MRT provide a very clear picture of the structure of the data and provide a highly intuitive insight into the kinds of interactions between variables (Crawley 2012). MRT result from the successive splitting of the sites (survey plots) into two groups based on a threshold from one of the explanatory variables, so that each group presents a minimum dissimilarity (De'ath 2002). The minimum number of observations to split (starting with n=129) was set to 20 and the minimum number of observations in a terminal node was set to 6. The MRT was built using the R packages *rpart* (Therneau et al. 2018) and *rpart.plot* (Milborrow 2017).

3.2.3.3 Occurrence of reproductive wildlings

This analysis aimed to understand the factors influencing the occurrence of reproductive wildlings. We used the proportion of reproductive wildlings in each plot as the response variable. Smaller wildlings (size 1 and 2; <1.3 m) were excluded from this analysis since only a very small percentage was found to be reproductive (<0.5% in both size classes). Therefore, our sampling units were the *inside* and *outside subplots* featuring size 3 (1.3-3 m) and/or size 4 (>3 m) wildlings.

We used a larger set of explanatory variables when compared to the previous analyses because the occurrence of reproductive eucalypt wildlings has been poorly investigated. Explanatory variables included: a) the set of site-scale variables used in previous analyses (see Section 3.2.2.1); b) the subplot location (*inside* or *outside subplots*); c) the number of wildlings of *size 3* and *size 4* (two variables); d) plantation-related variables, provided by Altri Florestal S.A., including the *plantation age* (time since planting), *plantation rotation* (first; second; third), the *stem age* (time since planting or coppicing) and *site index* (height of the dominant trees at ten years old); e) climate, altitude and soil variables, retrieved from APA (2018), namely: the *mean annual temperature* (ºC; average 1931-60), the *mean annual precipitation* (mm; average 1931-60), the *mean annual number of frost days* (average 1941-60), *altitude* (meters) and the *soil type* (cambisol; lithosol; luvisol).

In order to assess the factors influencing the proportion of reproductive wildlings, we used a generalised linear model (GLM) with a binomial distribution and logit link function. We computed VIF (>4) to check for collinearity between variables. Model simplification and validation followed the procedure previously described for the *presence* model (Section 3.2.3.1). Model fit was assessed using the proportion of explained deviance.

3.2.3.4 Distance of establishment from plantations

This analysis aimed to investigate the factors influencing the distance of establishment of *E. globulus* wildlings outside plantations. For this analysis, we used linear mixed models (LMM), with the *nlme* R package (Pinheiro et al. 2018). The dependent variable was the distance of wildling establishment from plantations. Since the dispersal distance of eucalypt wildlings has been less well investigated in eucalypt ecology, we included the same wider set of explanatory variables as in the analysis of reproductive wildlings (see Section 3.2.3.3), without the site-scale variables. Additionally, we included the variable *drainage line presence* (see Section 3.2.2.2) and variables retrieved from a digital terrain map: a) *transect aspect*, i.e. direction of exposure of that area (flat; four cardinal directions); b) *transect slope*, i.e. terrain inclination in that area (degrees). *Land cover* was not included in the analysis because some land cover types were underrepresented. Topographical variables (aspect; slope) were provided by Altri Florestal S.A. and originally retrieved from 1:25,000 cartography produced by the Portuguese Army Geographical Institute (IGeoE). Most transects were performed in downslope areas and *transect aspect* coincided roughly with the cardinal direction of the transect. The *transect* ID was included in the model as a random effect. We used VIF (>4) to discard correlated variables. Model simplification and validation followed the previously described procedure. As in the *presence* model (Section 3.2.3.1), model fit was assessed using the conditional R^2. All statistical analyses were performed using R software (R Core Team 2017).

3.3 Results

3.3.1 Wildling establishment along plantation edges

A total of 1,630 *E. globulus* wildlings were recorded in the 129 *presence plots*. Wildlings of *size 2* (0.5-1.3 m) were the most frequent (n=575; 35.3% of total), followed by *size 3* (1.3-3 m; n=481; 29.5%), *size 4* (>3 m; n=334; 20.5%) and *size 1* (< 0.5 m; n=240; 14.7%). The overall wildling density (all plants in total area of *presence plots*) was 1461.2 wildlings ha^{-1}, being higher in the *inside subplots* (1771.7 wildlings ha^{-1}) compared to the *outside subplots* (1044 wildlings ha^{-1}). Taking into account the factor wildling size, the overall wildling density was higher in the *inside subplots* for wildlings of *size 2* (650.5 vs. 334.0 wildlings ha^{-1}), *size 3* (556.7 vs. 262.6 wildlings ha^{-1}) and *size 4* (405.0 vs. 157.6 wildlings ha^{-1}), while it was lower for *size 1* wildlings (159.5 vs. 289.9 wildlings ha^{-1}), compared to the *outside subplots* (Fig. 3). The maximum wildling density was 6,000 wildlings ha^{-1}, registered in a 5x10 m *inside subplot*, composed exclusively of *size 1* (<0.5 m) wildlings. A paired Wilcoxon test showed that differences in wildling density between the *inside* and *outside subplots* were significant for wildlings of *size 2* (p=0.03), *size 3* (p<0.001) and *size 4* (p<0.001). We assessed the reproductive capacity of 1,820 neighbour *E. globulus* trees in the survey plots, of which 69% possessed capsules.

Fig. 2 - Reproductive *Eucalyptus globulus* wildlings of size 1 (<0.5 m) and size 2 (0.5-1.3 m). Red arrows indicate flowers

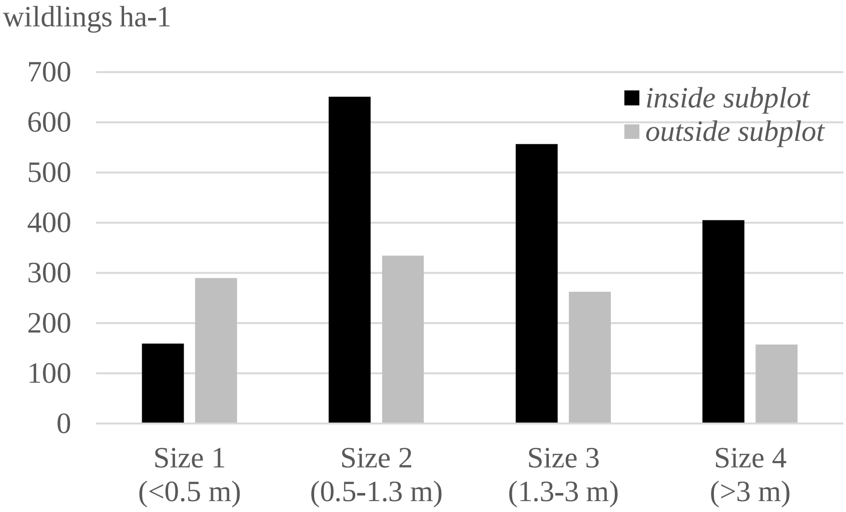

Fig. 3 - Overall density (all sampled plants / total surveyed area) of *Eucalyptus globulus* wildlings in the presence plots according to wildling size classes and subplot type

Among the 1,630 *E. globulus* wildlings found, there were 124 (7.6%) reproductive wildlings, i.e. sexually mature wildlings, possessing flowers or capsules. The overall density of reproductive wildlings was 115.6 wildlings ha^{-1}, being higher in the inside subplots (146.9 wildlings ha^{-1}) compared to the outside subplots (63.0 wildlings ha^{-1}). We found one reproductive *size 1* wildling (0.4% of the total *size 1* wildlings) and two reproductive *size 2* wildlings (0.3%; see Fig. 2). The proportion of reproductive wildlings was higher in *size 3* (5%; n=24) and *size 4* wildlings (29%; n=97).

3.3.1.1 Factors influencing wildling presence

The GLMM, performed to assess the influence of site-scale factors on wildling presence (Table 1), explained 32% of the variance. The variable *stem age*, included as a covariate, was retained in the model, but it was not significant. Three variables showed a significant, positive influence on wildling presence: *litter cover*, *plot slope* and *maximum capsule score*. The variable *edge aspect* also had a significant influence, particularly a negative effect of the *west* aspect on wildling presence.

Table 1 - Results from a binomial generalised linear mixed models (GLMM) produced to assess the influence of site-scale factors on the presence and absence of *Eucalyptus globulus* wildlings at the edges of plantations. The reference level of the categorical variable is shown in brackets

Variable	Coefficient	Std. Error	*Z* value	*P*-value
Intercept	-0.899	0.815	-1.102	ns
Stem age	-0.137	0.091	-1.498	ns
Litter cover	0.036	0.009	3.992	***
Plot slope	0.096	0.033	2.842	**
Max. caps. score	0.383	0.169	2.262	*
Edge aspect (*East*)				
North	-0.934	0.534	-1.749	ns
South	0.136	0.527	0.259	ns
West	-1.377	0.534	-2.579	**

Significance *P*-value: ≤0.001 = ***; ≤0.01 = **; ≤0.05 = *; >0.05 = ns

3.3.1.2 Factors influencing wildling abundance

The MRT, used to assess the influence of site-scale factors on the abundance of *size 1* (<0.5 m) wildlings in the *inside subplots* (see Section 3.2.3.2), created six clusters of plots. Four variables, mostly soil cover-related, were responsible for the five partitions originating the six clusters of plots (Fig. 4). Wildling density was found to be negatively related with *shrub cover* (first two partitions). The remaining plots were split by *stem age* of 10 years (third partition), where plots under trees with older *stem age* registered higher wildling density. The percentage of *bare ground* created the fourth partition, where plots featuring higher proportion of *bare ground* had higher wildling density. The fifth partition was created by the percentage of *herb cover*, being negatively related to wildling density. The whole tree explained 22 % of the variance.

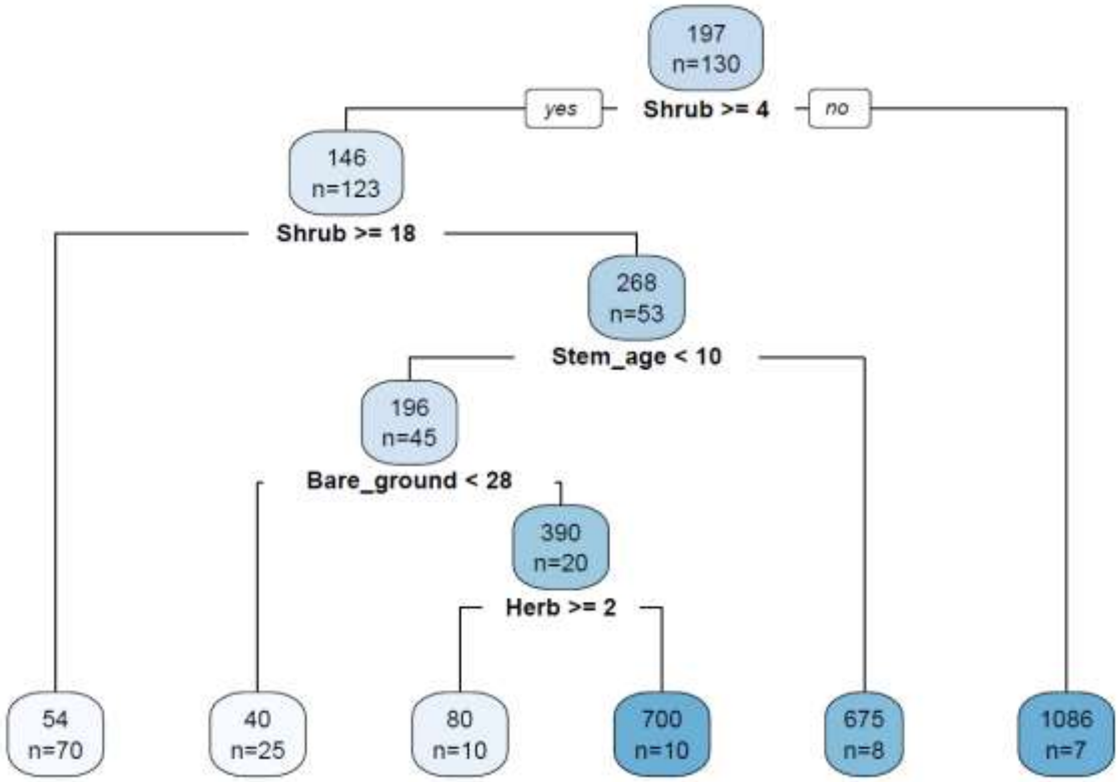

Fig. 4 - Multivariate Regression Tree (MRT) of *Eucalyptus globulus* wildling density (plants ha^{-1}; only wildlings up to 0.5 m tall – size class 1) using site-scale variables and stem age as explanatory variables (see Section 3.2.3.2). Each splitting node is represented by a box featuring the average wildling density (number above) and the corresponding number of plots (number below). Colour darkness in each box is proportional to the fitted value. The splitting variable and the threshold value responsible for the partition is presented below the box. The length of each branch is proportional to the difference in deviance between each node and the sums of the nodes below

3.3.1.3 Factors influencing reproductive wildlings

The final GLM produced to explore the factors influencing the proportion of reproductive *E. globulus* wildlings along the edge of plantations, retained 11 variables, but only seven were significant ($p<0.05$). The proportion of reproductive wildlings was positively influenced by the location in the *outside subplots*, a higher *site index*, *wet* aspects, high abundance of *size 4* (> 3m) wildlings and older *stem age* in the cultivated trees. On the contrary, higher *altitude* and increased *number of frost days* negatively affected the proportion of reproductive wildlings (Table 2). The model explained 53% of deviance.

3.3.2 Wildling establishment outside plantations

Most survey transects were established in *montado* (n=27; 55%) and shrubland (n=14; 29%). A few transects were established in paddock areas (n=5; 10%), and one transect (2%) was established in a pine stand, another in an agriculture area and another in a riparian area. We found a total of 641 *E. globulus* wildlings along the perpendicular transects, mostly established in *montado* (53.2%) and shrubland (39.9%). On average, we found 13.1 wildlings per transect (SD=14.6; min.=1; max.=70).

Around 75% of the *E. globulus* wildlings escaped from plantations were found within a distance of 15 m from the plantation edge, and c. 91% within 30 m. There were 18 wildlings (c. 3%) located beyond 50 m from the edge of plantations. The maximum distance recorded was 76 m. Around 59% of the *E. globulus* wildlings recorded outside plantations had adult foliage. The proportion of wildlings exhibiting adult foliage increased with the distance from the plantations (Fig. 5).

3.3.2.1 Factors influencing the distance of establishment

The LMM, computed to understand the factors influencing the dispersal distance of *E. globulus* from plantations, highlighted the importance of *drainage line presence*, the *soil type* and the *transect aspect* (Table 3). The presence of natural drainage lines along the surveyed area enhanced the distance of wildling establishment. On the other hand, the distance of establishment was negatively influenced by west exposures and, with less importance, by the occurrence of podzols. The fitted model with these three variables explained 18% (Nagelkerke R^2) of the observed variance.

Table 2 - Results of a generalised linear model (GLM) used to assess the factors influencing the proportion of reproductive *Eucalyptus globulus* wildlings along the edges of plantations. The reference level of categorical variables is shown in brackets

Variable	Coefficient	Std. Error	*Z* value	*P*-value
Intercept	11.380	2.233	-5.094	***
Subplot location				
(*Inside subplot*)				
Outside subplot	0.824	0.255	3.222	**
Bare ground	0.016	0.009	1.801	ns
Shrub cover	0.012	0.006	1.880	ns
Site index	0.232	0.085	2.727	**
Soil type				
(*Cambisols*)				
Lithosols	-15.590	911.900	-0.017	ns
Podzols	0.571	0.333	1.715	ns
Altitude	-0.008	0.002	-3.822	***
Plot aspect				
(*Dry*)				
Wet	0.577	0.265	2.176	*
Plot topography				
(*Flat*)				
Ridge	-17.470	1,859.000	-0.009	ns
Slope	0.032	0.279	0.115	ns
Valley	-0.504	0.546	-0.922	ns
Wildling size				
(*Size 3*; 1.3-3 m)				
Size 4; >3 m	0.152	0.024	6.277	***
Stem age	0.600	0.113	5.279	***
Annual number frost days	-0.272	0.081	-3.343	***

Significance *P*-value: ≤0.001 = ***; ≤0.01 = **; ≤0.05 = *; >0.05 = ns

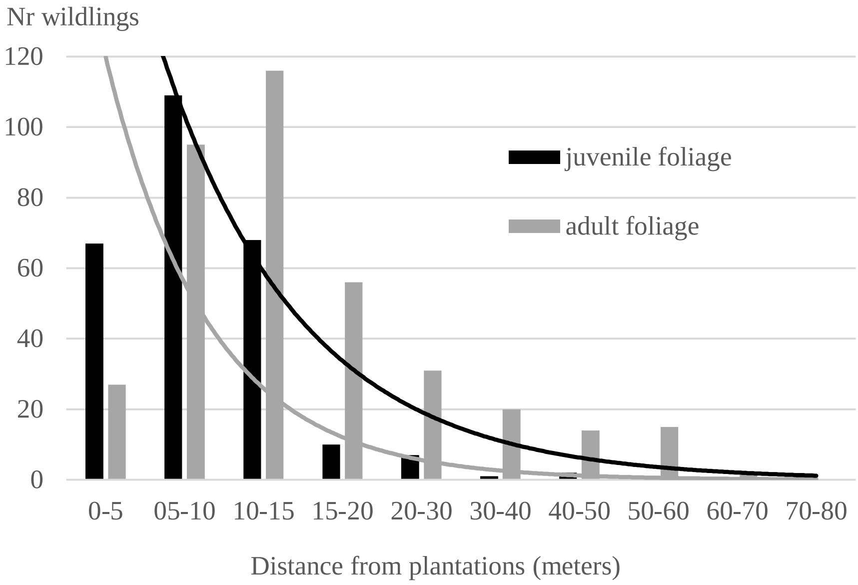

Fig. 5 - Number of *Eucalyptus globulus* wildlings with juvenile and adult foliage according to distance from the plantation. The general decreasing trend is illustrated using fitted negative exponential curves

3.4 Discussion

3.4.1 Wildling establishment along plantation edges

Most previous studies investigating the natural establishment of exotic eucalypts do not consider wildling development, especially the onset of sexual reproduction. This study provides evidence of the effective recruitment of *E. globulus* wildlings along the edges of plantations. Others have reported a high rate of mortality of *E. globulus* wildlings in the first months after germination (Fernandes et al. 2017). Calviño-Cancela and Rubido-Bará (2013) found that the survival probability of *E. globulus* seedlings increases every month, reaching 82% between the 6th and 8th months. We found no previous information regarding the growth rate of naturally established *E. globulus* wildlings. However, based on recent field observations in Portugal, we are confident that wildlings of *size 3* (1.3-3 m) and *size 4* (>3 m), which comprise half of the surveyed wildlings in this study, should be more than six months old, and thus have high chances of survival.

Table 3 - Results from a linear mixed model (LMM) showing the variables influencing the dispersal distance of *Eucalyptus globulus* from plantations into adjacent areas. The reference level of categorical variables is shown in brackets

Variable	Coefficients	Std. Error	*t*	*P*-value
Intercept	20.694	3.450	5.600	***
Drainage line				
(Absence)				
Presence	18.350	1.910	9.609	***
Transect aspect				
(East)				
West	-9.9202	3.392	2.924	**
North	-5.281	3.586	-1.473	ns
Flat	-3.160	3.062	-1.032	ns
South	3.398	3.542	0.959	ns
Soil type				
(Cambisols)				
Podzols	-7.177	3.127	-2.295	*
Lithosols	4.070	4.798	0.848	ns

Significance *P*-value: ≤0.001 = ***; ≤0.01 = **; ≤0.05 = *; >0.05 = ns

We recorded higher wildling abundance in the *inside subplots*. A similar trend was found in other studies (Águas et al. 2017; Fernandes et al. 2016; Larcombe et al. 2013). This discrepancy between *inside* and *outside subplots* is probably related to the higher frequency of disturbances outside plantations, such as the passage of vehicles and people, or fuel reduction operations for fire prevention, targeting especially larger plants, as hypothesised by other studies (Águas et al. 2017; Larcombe et al. 2013). This discrepancy between *inside* and *outside subplots* is more significant for older plants (>50 cm; sizes 2, 3 and 4). This is consistent with the findings of a similarly study in Australia by Larcombe et al. (2013), and seems to support the idea that disturbances such as multi-year management cycles or stochastic events outside the plantation may periodically reduce abundance.

3.4.2 The role of site-scale factors on wildling establishment

The *presence* of *E. globulus* wildlings along the edge of plantations was affected by factors as diverse as soil cover, topography, propagule pressure and aspect. We note that because *presence* considered wildlings of all sizes, some more dynamic site-scale variables, such as *soil cover* and *maximum capsule score*, may not portray the conditions at the time of wildling establishment.

Among the soil cover variables, *litter* positively affected wildling presence, being the most significant variable. We did not discriminate the type of litter but it included mostly eucalypt leaves, branches and bark, even though litter from other plant species was also common and, in a few locations, quite conspicuous. The amount of litter may reflect indirectly important factors for wildling establishment. For instance, litter is positively related to the residence time of the plantations, which in its turn indicates a greater propagule pressure and thus more chances of wildling establishment. More litter also suggests that the plantation had not been recently managed, therefore favouring wildling persistence. Likewise, more litter should correspond to lower cover of other categories, in particular shrubs or herbs which compete for light and soil resources, and thus may limit the establishment of *E. globulus* wildlings, which are particularly sensitive to competition (Kirkpatrick 1977). In fact, it is known that *E. globulus* litter causes negative allelopathic effects on other plant species (Souto et al. 2001; Souto et al. 1994).

Plot slope was also found to affect positively the presence of *E. globulus* wildlings. Catry et al. (2015) found a similar result in roadsides and hypothesised that, in steeper areas, plant competition is lower due to greater soil erosion, and there is lower likelihood of waterlogging. In our case, it is also likely that areas featuring steeper slopes are managed less frequently or less effectively in terms of vegetation removal, due to technical and operational limitations.

The *maximum capsule score* (an indicator of reproductive output) was a significant factor positively affecting the presence of *E. globulus* wildlings, which is consistent with the findings of a similar study in Australia by Larcombe et al. (2013). It is known that propagule pressure has a central importance in plant invasions (Simberloff 2009). In our study, locations where trees produce more flowers and seeds are also likely to have wildlings.

The *west exposure* of the edge of the plantation, was the only site-scale factor influencing negatively the presence of *E. globulus* wildlings. Winds in this region are predominantly from northwest, meaning that west edges are more frequently exposed to wind. Therefore, one explanation may be related with higher seed-shadow drift towards the inner part of the stand, resulting in lower wildling density close to the edge of the plantation. Another possible explanation may be that western plots have no shelter against predominant winds, which may act together with higher sunlight exposure and higher temperatures, resulting in higher drieness and therefore worst conditions for wildling establishment (Stoneman 1994; Wang et al. 1998).

The analysis of wildling abundance, using Multivariate Regression Trees (MRT), aimed to detect direct correspondence between local conditions and the abundance of young plants (<0.5 m; see Section 3.2.3.2). We found that *shrub cover*, *bare ground cover* and *herb cover*, were responsible for four of the five data partitions in the MRT. These results seem to highlight the importance of competition with other vegetation in the emergence and development of *E. globulus* wildlings, because shrubs and herbs negatively affected wildling density, while bare ground had the opposite effect. The findings are also in line with the known preference of eucalypt wildlings for disturbed, competition-free areas (Fernandes et al. 2018; Kirkpatrick 1977). The positive effect of *stem age* on wildling abundance should reflect the positive influence of residence time and the increasing propagule pressure originating from the cultivated trees as they grow older (Lockwood et al. 2005; Richardson 1998; Simberloff 2009).

3.4.3 Reproductive offspring

To our knowledge, this is the first study to record and assess the reproductive status of *E. globulus* wildlings. Reproductive *E. globulus* wildlings were not found in a similar survey conducted on *E. globulus* plantations in Australia (Larcombe et al. 2013). Importantly, flowering was identified in juvenile wildlings (Fig. 2), which does not normally occur until after the transition to adult leaves (Potts & Gore 1995). Early flowering is often a target of breeding programs (Flachowsky et al. 2011), and may have been specifically targeted to accelerate production during the development of the *E. globulus* industry in Portugal. Time to first flowering is under strong genetic control in *E. globulus* (Jordan et al. 1999) and is highly heritable (Chambers et al. 1997). A short juvenile period was also found for *E. grandis* in South Africa (Musengi & Archibald 2017) and is a common trait among some of the most invasive plant species (Grotkopp et al. 2002; Rejmánek & Richardson 1996).

Among the factors influencing the proportion of reproductive wildlings, wildling size (height) was the most significant. Larger wildlings (*size class 4*; >3 m) were more likely to be reproductive, which is similar to observations in South Africa for *E. grandis* wildlings (Musengi & Archibald 2017). *Stem age* was the second most influential factor, also showing a positive influence. Again, older plantations have more chance of accommodating older (larger) wildlings, which in its turn have a greater likelihood of being reproductive.

The *number of frost days* negatively affected the proportion of reproductive wildlings. Countrywide surveys in Portugal suggest that higher numbers of frost days limits wildling abundance (Catry et al. 2015; Deus et al. 2016). This has been associated with the sensitivity of wildlings to frost and freezing temperatures, but recurrent frost may also supress flowering or it may damage the reproductive structures of plants. Likewise, *altitude* was found to affect negatively the proportion of reproductive wildlings, despite a short altitudinal range (30 to 307 m) in our sampling areas. Altitude may have acted as a surrogate of local edapho-climatic conditions as hypothesised by Catry et al. (2015). In fact, a posterior analysis revealed that the sampling areas at higher altitudes are also located towards inland, probably experiencing lower temperatures and higher thermal amplitudes, which may supress flowering.

Being in the *outside subplots* increased the chances of *E. globulus* wildlings being reproductive. This probably reflects lower competition for light and nutrients, which may enhance flowering (Williams et al. 2006), meaning that wildlings on the edges of plantations and those dispersed into adjacent open areas may be more likely to be reproductive. This may be corroborated by findings in Australia, where greater abundance of flowering *E. globulus* trees was found in the edges of plantations, when compared to the trees inside plantations (Barbour et al. 2008), and where lower tree densities in plantations promoted flowering in the related *E. nitens* (Williams et al. 2006). *Site index*, which is an indicator of site productivity, also increased the proportion of reproductive wildlings. It is probable that the same site conditions that enhance the growth of cultivated trees (nutrients, moisture), also favour the development of reproductive organs in younger plants.

3.4.4 Wildling establishment outside plantations

Survey transects outside plantations were established in different land cover types, indicating that *E. globulus* can spread into different habitats. *Land cover* effect was not analysed, because some land cover types were underrepresented. The unequal distribution of land covers could be indicative of different levels of susceptibility to invasion or an unequal representation of the areas surrounding *E. globulus* in the study region. Nonetheless, other studies found that some habitats such as native forests and grasslands are more resistant to *E. globulus* invasion (Calviño-Cancela & Rubido-Bará 2013; Fernandes et al. 2016). In our study, most transects and most of the surveyed wildlings were established in *montado*, a common agroforestry system in the region characterised by an open forest of native oaks.

Our survey protocol was biased towards finding *E. globulus* wildlings outside plantations, because the survey transects were triggered by the existence of at least one wildling at a minimum distance of 10 m. However, a recent study conducted in Central and North Portugal, using random survey transects outside *E. globulus* plantations, found wildlings in nearly one third of the transects (Fernandes et al. 2016), indicating that this is not a rare event.

In our study, we found *E. globulus* wildlings established up to a distance of 76 m, similarly to observations by Fernandes et al. (2016) in Portugal. These dispersal distances are consistent with the maximum seed dispersal distance from *E. globulus* plantations recorded in NW Spain (80 m; Calviño-Cancela & Rubido-Bará 2013). The sharp decrease in the number of *E. globulus* wildlings after 15 m was also observed in other studies investigating the escape of *E. globulus* (Fernandes et al. 2016; Larcombe et al. 2013) and other *Eucalyptus* spp. (Callaham Jr et al. 2013). Seeds of *E. globulus* do not have any particular adaptation for seed dispersal and most seeds are dispersed by gravity and wind (Cremer 1965, 1977). Calviño-Cancela and Rubido-Bará (2013) found that practically all *E. globulus* seeds (98.6%) fall within 15 m from the edge of plantations. Nevertheless, in this study, we found a larger proportion of wildlings at greater distances than in previous studies, which can be explained by our survey protocol. Instead of using survey plots along the transects outside plantations (e.g. Fernandes et al. 2016), we opted for a visual detection on both sides of the transect. As a result, we recorded more *E. globulus* wildlings than we would if we opted for survey plots. Our survey protocol should also explain why the proportion of adult *E. globulus* wildlings also increased with distance from the plantation, because larger wildlings are more easily detectable, while smaller wildlings likely remained unnoticed when distanced more than a few meters from the transect. It is important to stress that many of the surveyed wildlings established outside plantations possessed adult foliage, meaning that they overcame the most critical stage of survival and reached adulthood. This transition is recognised as a critical step in plant invasions (Richardson et al. 2000). Escaped wildlings can act as a stepping-stone for posterior spread in the absence of management (Moody & Mack 1988; Richardson et al. 1994).

The factors influencing the distance of establishment of *E. globulus* from plantations highlighted the importance of the main vectors of seed dispersal: wind and water. Wind is considered the main vector of dispersal of eucalypts (Cremer 1977). The distance of wildling establishment was inferior in west exposure when compared to east, in line with the dominant winds in this region, which are predominantly from northwest. The same trend was observed in California, where the greatest dispersal distances of *E. globulus* wildlings coincided with the direction of the prevailing winds (McBride et al. 1988).

Water is also acknowledged as an important dispersal vector of eucalypt seeds (Forsyth et al. 2004; Kirkpatrick 1977). In our study, the presence of a natural drainage line was found to increase the distance of establishment of *E. globulus* from plantations suggesting that hydrochory may play an important role in wildling dispersal. In Australia, Larcombe et al. (2013) suggested that a drainage line might be responsible for the establishment of an *E. globulus* wildling at a distance of 175 m from a plantation. Apart from wind and water, ants may also contribute to the secondary dispersal of *E. globulus* seeds in Portugal (Deus et al. 2018b). Our analysis revealed that podzols affected negatively the distance of establishment from plantations. Soil itself has no influence on seed dispersal but it may affect wildling establishment. A study conducted in Portugal reported the opposite effect, finding that podzols positively influenced wildling abundance (Catry et al. 2015). We suspect that the effect of soil type maybe an artefact of the analysis related to other important factors such as slope and land cover.

3.5 Conclusions

The survey along the edges of *Eucalyptus globulus* plantations in Central Portugal provided evidence of an effective wildling recruitment since many wildlings overcame the most critical period for survival. The abundance of young *E. globulus* wildlings was mostly affected by soil cover variables symptomatic of the harmful impact of plant competition. Wildling presence was influenced by a wider range of site-scale factors including slope, aspect of the plantation edge and propagule pressure. Around 8% of the surveyed wildlings reached sexual maturity, supporting the hypothesis that the species is naturalised. A few very young wildlings were reproductive suggesting that the species can have a very short juvenile period. The proportion of reproductive wildlings was related to different factors ranging from plant development (positively related to wildling size), plantation-related (positively related to stem age and site productivity) and climatic variables (negatively related to the number of frost days).

This study also provides evidence of an effective recruitment of *E. globulus* outside plantations, in different habitats, since more than half of the surveyed wildlings outside plantations were adult. Wildlings were found up to 76 m of distance. Greater dispersal distances coincided roughly with the direction of the prevailing winds and the presence of natural drainage lines.

This study tackled important knowledge gaps about the demography, phenology and ecology of *E. globulus* in the introduced range, which may help to identify the factors and situations enhancing *E. globulus* recruitment and escape from plantations. The development of reproductive wildlings can increase propagule pressure from plantation edges and outside plantations they can act as a stepping-stone for posterior spread in the absence of management. Particular caution is needed in downslope aligned with predominant winds and with the presence of drainage lines capable of significantly enhancing seed dispersal. Further research is needed to evaluate the reproductive capacity of *E. globulus* wildlings escaped from plantations, the parental relationships among naturally established populations outside plantations, the rate of expansion and the ecological succession of invaded areas.

3.6 References

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Chapter 4: The effect of management operations on the demography of *Eucalyptus globulus* seedlings

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https://www.dropbox.com/s/88tndpn9vo6boq8/Chapter3SuppMaterial.pdf?dl=0

4.1 Introduction

Eucalyptus globulus Labill., native to south-eastern Australia, Bass Strait Islands and Tasmania (Jordan et al. 1994), was the first eucalypt species to be extensively cultivated outside the native range (Jacobs 1979). Plantations of *E. globulus* can currently be found in countries such as Portugal, Spain, USA, Chile, Uruguay, China, Australia and Ethiopia (Potts et al. 2004). Portugal is currently the country with the largest area occupied by *E. globulus* plantations, covering more than 800,000 ha, the equivalent to c. 9% of the country's mainland surface (ICNF 2013).

There has been an increased concern about the capacity of *E. globulus* to naturalize and produce offspring, within and around planted areas. There are records of invasive behavior in different regions of the world (Rejmánek & Richardson 2013). In Central California, Fork et al. (2015) estimated with aerial images that the expansion rate of six *E. globulus* stands was 271% between 1931 and 2011. The same invasive behavior was found in Angel Island also in California (McBride et al. 1988). In South Africa most works on eucalypt invasiveness are about *E. camaldulensis* (e.g. Tererai et al. 2015). Also in South Africa, surveys recorded outside *Eucalyptus grandis* plantations showed wilding densities between 670 and 1,000 wildlings ha^{-1} (Musengi & Archibald 2017). These wildings showed a wide range of wildling sizes (max. 30m tall), suggesting continuous recruitment along time or the occurrence of several successful recruitment events.

In Portugal, *E. globulus* recruitment occurs across a wide range of environmental conditions (Catry et al. 2015), both inside (Águas et al. 2014; Vaz et al. 2019) and outside (Deus et al. 2019; Fernandes et al. 2016) plantations. In the introduced range seedling recruitment is usually unwanted for economic and environmental reasons, as it causes negative impacts on biodiversity (Richardson 1998), plantation productivity (Rejmánek & Richardson 2011) and fire hazard due to increased fuel load and continuity (Calviño-Cancela et al. 2016). Therefore, it is of major importance to understand the drivers and factors influencing *E. globulus* recruitment.

Much research was aimed at understanding and assessing *E. globulus* recruitment and invasiveness, in Portugal (Deus et al. 2019), Spain (Calviño-Cancela et al. 2018), Australia (Larcombe et al. 2013) and Chile (Becerra & Bustamante 2011). Previous research shed light into some key factors that may influence *E. globulus* recruitment, such as climate (Catry et al. 2015; Deus et al. 2019), fire (Águas et al. 2014; Calviño-Cancela et al. 2018), plantation characteristics (Águas et al. 2017; Deus et al. 2019) and site characteristics (Becerra & Bustamante 2011; Calviño-Cancela & Rubido-Bará 2013; Fernandes et al. 2017; Fernandes et al. 2018).

Most research on eucalypt recruitment and invasiveness has been based on field surveys commenced after most of the detected plants had overcome the most critical period of survival (Catry et al. 2015; Deus et al. 2019). Using sowing experiments, a few studies investigated the germination and early demography of *E. globulus* seedlings in field conditions. These studies showed that the germination and the survival of newly germinated *E. globulus* were influenced by factors as diverse as habitat, shadow, climate, sowing season and fire (Becerra & Bustamante 2011; Calviño-Cancela et al. 2018; Calviño-Cancela & Rubido-Bará 2013; Fernandes et al. 2017).

Among the factors influencing *E. globulus* recruitment, disturbance of soil and vegetation were revealed to be paramount particularly due to the temporary reduction or elimination of plant competition (Calviño-Cancela et al. 2018; Fernandes et al. 2017; Fernandes et al. 2018). In the native range, disturbance is one of the key factors of eucalypt establishment (Stoneman 1994). The same is true in the introduced range. For instance, in California, recruitment occurs only where and when competing vegetation is absent (Kirkpatrick 1977). In Brazil, eucalypt recruitment occurs in bare soil gaps adjacent to plantations (Miolaro et al. 2017). In Portugal, *E. globulus* recruitment is positively related to the level of disturbance (Fernandes et al. 2018). Another poorly studied factor affecting recruitment is seed predation. In the native range, eucalypt seeds are heavily predated by ants (Andersen & Ashton 1985; Ashton 1979). In northwest Spain, in the context of a sowing experiment, a preliminary study with *E. globulus* showed no evidence of seed predation (Calviño-Cancela & Rubido-Bará 2013). However, a recent study confirmed that *E. globulus* seeds are predated in Portugal (Deus et al. 2018).

Wildfires are major disturbance events enhancing *E. globulus* recruitment (Águas et al. 2014; Calviño-Cancela et al. 2018). Marked disturbance is also caused by different management operations often performed within and around *E. globulus* plantations. Management operations in forested areas include techniques such as harrowing, prescribed fire and soil stripping, aimed at different objectives. *S*oil harrowing is performed between the rows of *E. globulus* plantations to reduce fuel load (lowering competition and fire hazard) and improve general soil conditions (Carneiro et al. 2008; Gonçalves et al. 2008; Mirra et al. 2017). Prescribed fire is an alternative to reduce fuel loads in *E. globulus* plantations (Mirra et al. 2017; Pinto et al. 2014), even though it is more frequently applied outside plantations. Soil stripping, i.e., soil levelling with a blade, is frequently used to create or maintain dirt roads, roadsides and firebreaks adjacent to plantations.

Each type of management operation causes different physical and chemical modifications of the soil (Madeira et al. 1989; Pagliai et al. 2004), affecting biotic and abiotic conditions that may influence *E. globulus* recruitment. For instance, apart from the mechanical destruction of most of the vegetation, soil harrowing and stripping affects soil structure in different ways (e.g., porosity and compaction) and its micro-topography (irregular or flat surface). It is known that microsite features (e.g., slope; aspect; depressions; sheltering objects) favour the retention of moisture and facilitate eucalypt recruitment (Bailey et al. 2012; Battaglia & Reid 1993). Likewise, prescribed fire may modify soil chemistry, which can also affect the germination and early development of *E. globulus* (Águas et al. 2018). Despite their differences, most management operations result in bare ground areas where eucalypt recruitment is enhanced (Fernandes et al. 2018; Kirkpatrick 1977).

Management operations will likely destroy most *E. globulus* seedlings, but the effect on propagules (capsules and seeds) is not as obvious. For instance, if the soil is harrowed, burying capsules and the small eucalypt seeds greater than a few centimetres may prevent emergence (Cremer 1965; Free 1951). Likewise, fire may have a destructive impact on seeds and capsules on the ground (Santos et al. 2015; Silva et al. 2016). However, if seeds are dispersed from canopy-stored seed after such treatments, they may find the right conditions to germinate and survive, depending on the site conditions and weather. Small seedlings of *E. globulus* are known to be extremely sensitive to extreme conditions, such as water stress or cold temperatures (Jacobs 1979; Stoneman 1994). Low temperatures limit the establishment of eucalypts worldwide (Booth 2012; Jacobs 1979). Freezing can severely damage the tissues of *E. globulus* seedlings, and temperatures around 0ºC were enough to affect the water uptake and growth of seedlings (Almeida et al. 1994). Other several studies have shown that climate plays a key role in the mortality of young *E. globulus* seedlings (Calviño-Cancela & Rubido-Bará 2013; Fernandes et al. 2017). However, management operations can influence, indirectly, the effect of weather by creating micro-niches for seedling establishment, such as sites with a shadow or favouring moisture accumulation, and affecting the soil water repellence (Bailey et al. 2012; Doerr & Thomas 2000; Ferreira et al. 2000; Madeira et al. 1989).

Despite the existing research on *E. globulus* recruitment and invasiveness, the impact of disturbances caused by standard management operations remains poorly explored. Little is known about the impact of different management operations on the propagules of *E. globulus* (capsules and seeds), scattered in the soil surface either before or after these treatments, and the subsequent effects on the germination and early demography of *E. globulus* seedlings. In addition, little is known about the influence of weather variables on the demography of newly-germinated *E. globulus* seedlings across time.

The present study aims at assessing the effects of standard management operations and weather on the germination and early demography of *E. globulus* seedlings. It investigates the role of capsules as opposed to free seeds in *E. globulus* establishment, and to compares the influence of different management operations on pre and post-dispersed propagules. With this purpose, a sowing experiment was conducted in Portugal, using different treatments related to the type of management operations, the sowing time, *before* or *after* management, and the type of dispersal, through *seeds* or *capsules*. Specific objectives were to assess: a) the role of different management operations on seed germination and survival of *E. globulus* seedlings; b) whether the effects of these treatments as dependent upon whether propagules were dispersed *before* or *after* these operations; c) the importance of *capsule* dispersal, compared to free *seed* dispersal; and d) the influence of weather variables on seedling survival across time. Additionally, we established a small seed predation experiment in order to check for the existence of seed-animal interactions in the area.

4.2 Methods

4.2.1 Study species

Eucalyptus globulus, commonly known as Tasmanian blue gum, is an evergreen broadleaf tree belonging to the Myrtaceae family, reaching up to 45-55 m height (Goes 1984; Jacobs 1979). It bears serotinous pseudo-capsules that protect the seeds, until they dry-out, allowing seed shed. Capsule valves open after tissue necrosis, namely after the fire, and release the seeds (Silva et al. 2016). Each capsule may contain approximately 6-25 viable seeds (Hardner & Potts 1995), although this number is highly variable. As with most eucalypts (Booth 2017), *E. globulus* seeds do not have a specialized dispersal mechanism

(Kirkpatrick 1977), but gravity combined with wind are considered to be the main dispersal drivers (Calviño-Cancela & Rubido-Bará 2013; Deus et al. 2019). Seed dehiscence occurs throughout the year, but peaks usually occur after hot periods or wildfires (Cremer 1965; Santos et al. 2015). Seeds germinate readily after being exposed to moisture owing to a thin testa and lack of an endosperm (Larcombe et al. 2013).

4.2.2 Experimental design

In order to assess the impact of different management operations on the germination and early demography of *E. globulus* seedlings, we conducted a sowing experiment using *E. globulus* seeds and seed-filled capsules. The experiment was conducted at the campus of the College of Agriculture of Coimbra. An experimental sowing field (170 m x 36 m) was established on a flat stream bank covered three years before by homogeneous mineral soil originated from excavations for road building (coordinates: 40°12'16.6"N; 8°27'11.4"W). Laboratory analyses (not presented in this study) revealed that the soil was relatively homogeneous, with very low levels of organic matter (less than 2%).

The sowing field was divided into 10 blocks (34 m x 18 m). Each block contained 16 plots corresponding to 16 specific treatment combinations resulting from a full factorial design with three factors: 1) the type of management operations (*burning*, superficial soil *harrowing*, soil *stripping* and a *control*); 2) the sowing time (sowing *before* or *after* operations); and 3) the sowing type (two levels: sowing of *seeds* or *capsules*). Each treatment was thus replicated 10 times (once in each blocks), giving a total of 160 sampling units (16 treatments x 10 blocks/replications). Each management operation (*burning*, *harrowing*, *stripping*, *control*) was applied in a plot measuring 8.5 m length and either 6 m or 3 m wide, depending on the type of management operation. For operational reasons, the plots subject to operations using a tractor (soil *harrowing* and *stripping*) were wider (6 m wide), while *burn* and *control* treatments were confined to narrower plots (3 m wide).

In the centre of each plot, we established a 0.5 x 0.5 m subplot where seeds or capsules were sown either *before* or *after* the management operations. In the plots adjacent to the *burning* operations, the subplot was established at a greater distance (4.5 m) from the fire to avoid effects of the radiating heat, even though fire intensity was relatively low. A scheme of the experimental design is available in Supplementary material (Fig. S1).

4.2.3 Sowing, management operations and monitoring

Seeds of *E. globulus* used in the experiment were from a single provenance of commercial stock, provided by the pulp company Altri Florestal S.A. (Altri). Germinability of this seed lot was 90% (laboratory tests). The seeds were kept under controlled conditions (around 5ºC and 20% moisture) until sowing. Capsules used in the experiment were collected seven days before sowing from a single mother-tree in an Altri seed orchard, to keep genetic provenance as homogenous as possible. Capsules were left to dry, upright, at room environment in order to be dehiscent (open valves) at sowing time. Germinability of encapsulated seeds was 86% (laboratory tests). On average, each capsule had 43.2 seeds (n=10; SD = 12.6; min. = 25; max. = 62).

Sowing of seeds and capsules was performed in late September 2013. This period was chosen because it corresponds to a typical dehiscence period of *E. globulus* seeds, following hot periods or wildfires, which usually occur in July-September. In the subplots sown with *seeds* (50%; n = 80), a total of 100 seeds were manually dispersed in each 0.5 x 0.5 m subplot, trying to simulate natural seed rain (note that seeds were not buried). Similar seed densities were used in other studies (Calviño-Cancela & Rubido-Bará 2013). In the subplots sowed with capsules (50%; n = 80), a total of 10 capsules were dropped in each subplot. Capsules were dropped from an approximate height of 1.70 - 1.80 m. A total of 8000 seeds and 800 capsules of *E. globulus* were sowed in this experiment. Based on the average number of seeds per capsule, we estimate a total of 34,560 encapsulated seeds (800 capsules x 43.2 seeds) sowed in the *capsule* plots.

Sowing of *E. globulus* seeds and capsules, as well as all management operations (*harrowing*; *stripping*; *burning*), occurred between the 18th and 27th of September 2013. In the *control* plots, vegetation was left undisturbed (no management). Nevertheless, the same number of plots, as in other management operations, was assigned as *before* (n = 20) or *after* (n = 20), and seeds and capsules were sowed in the same days as in other *before* and *after* plots.

Soil *harrowing* was performed with a heavy disk harrow pulled by a tractor. *Stripping* was done with a tractor equipped with a levelling blade (see photos in Supplementary Material: Fig. S3; Fig. S4). In order to avoid contamination between plots, by dragging seeds or capsules between adjacent plots through soil harrowing or stripping, the operation was interrupted at the end of each plot. The experimental burn was conducted as a head fire (wind direction), under moderate weather conditions (average temperature = 25ºC; average wind speed = 8 km/h; average humidity = 51%). The weather was monitored in real-time by a portable weather station. Fuels were mostly dry herbaceous plants. Flame length ranged between 0.5 m and 1.5 m. The burn operation was conducted using drip torches.

4.2.4 Data collection

Monitoring of the subplots was performed weekly after sowing, until the number of germinations of *E. globulus* seedlings declined to nearly zero, approximately two months after germination. Monitoring was done every two weeks thereafter, until the end of the experiment, approximately one year after sowing (September 2014). During that year, we did a total of 32 monitoring visits. The same researcher made all the visits during the experiment. In each visit, in each sowing subplot, the researcher registered the number of germinations, the number of deaths and the number of surviving *E. globulus* seedlings. Germinations (new plants) were marked with white wire, in order to distinguish the new recruits in the next visit. Dead plants were removed right after being detected. The search for *E. globulus* seedlings was also sporadically performed outside the sowing subplot, particularly in the plots subject to soil *harrowing* and *stripping*, in order to detect seedlings originating from seeds dragged by those treatments. There was no individual tagging of seedlings, i.e., plants did not have individual identifications. Therefore, data were analysed at the plot- not - plant-level.

A weather station located at the campus of the College of Agriculture of Coimbra provided meteorological data daily. The following data were retrieved for each day of the experiment (duration of 1 year): *maximum*, *minimum* and *average temperatures* (ºC); *precipitation* (mm); relative *humidity* (%); *average wind speed* (km h^{-1}); and *solar radiation* (MJ m^{-2}). We computed *crop evapotranspiration* (ETc) based on the potential evapotranspiration (ETo), obtained by the Penman-Monteith equation (Allen et al. 1998). *Crop evapotranspiration* was computed using the dual crop coefficient methodology (Allen et al. 1998; Allen et al. 2005), which separates crop transpiration, from soil water evaporation. Additionally, we computed the soil water balance in the root zone in order to estimate the *soil water content* available for plant growth (Allen et al. 1998). The values of soil water content at field capacity and wilting point were estimated as 26.5% and 12.5%, respectively. A critical soil water content of 19.5% was assumed, corresponding to a minimum content to assure water comfort conditions for seedlings. This value was obtained considering that half of the total available water is easily or readily available for evapotranspiration (González et al. 2015). A surface soil layer of 20 cm was assumed in the water balance.

4.2.5 Seed predation experiment

At the time of the experiment, it was unknown if *E. globulus* seeds shed on the ground were likely to be predated by animals, thus influencing germination rates by reducing the number of available seeds. During fieldwork, we observed animal activity, such as rodent faeces and foraging by some ant colonies across the experimental field. Therefore, in order to assess the possible influence of seed predation in our sowing experiment, we performed a small experiment to test the existence of predation of *E. globulus* seeds in the area.

We used Petri dishes (c. 10 cm diameter and 1 cm deep) as animal feeders. In order to distinguish the animal types accessing the seeds, and to control for external factors (wind and rain) in seed removal, we created different animal exclusion treatments: vertebrate exclusion, invertebrate exclusion, no exclusion and total exclusion (*control*). See details about feeder types in (Deus et al. 2018). In an area adjacent to the experimental field, we established three groups of feeders distanced 10 meters from each other, each group comprising one each of the four different feeder types. Inside each feeder, we placed five seeds of *E. globulus*. For comparison purposes, we added in each feeder five seeds of the native shrub *Cistus salviifolius* L. (similar-sized seeds) and five seeds of the alien, myrmecochorous *Acacia* spp. (a mix of *A. dealbata* Link and *A. saligna* (Labill.) H. L. Wendl.). Each day we counted the number of seeds of each species that were removed or eaten, and replaced the missing or eaten seeds by fresh seeds of the same species. Feeders were monitored for seven days, between the 4th and the 10th of January 2014.

4.2.6 Data analysis

In order to understand the overall effects of the three study factors on the number of *E. globulus* seeds germinated, we used Multivariate Regression Trees (MRT). MRT result from the successive splitting of the sampling units (plots) in two groups. Each split is driven by a splitting rule based on the most influential explanatory variable, so that each group (or branch) of samples presents minimum dissimilarity (De'ath 2002). The MRT approach was preferred to a GLM approach due to the overwhelming influence of the *sowing type* and the *sowing time* factors. The MRT analysis was performed using the R packages *rpart* and *rpart.plot* (Therneau et al. 2018) using the three study factors as explanatory variables (n = 160).

Since most of the plots registered no germinations (59.4%), especially plots sowed with capsules (only two out of 80), we performed a second analysis considering only seed-sown plots with at least one germination, to allow testing of significance of the differences among the different treatments. In this case we used Generalised Linear Mixed Models (GLMM) to assess the role of *management operations*, *sowing time,* and their interaction on the number of germinations. The model was built using a Poisson error distribution and using *replication* as a random effect. The GLMM was performed using the R package *lme4* (Bates et al. 2017). Model overdispersion was assessed using the function *overdisp_fun* from the same package (Thomas et al. 2015). The variance explained by the model was estimated using the R package *MuMIn*, according to the method proposed by (Nakagawa & Schielzeth 2013).

In order to assess the influence of *management operations* on *E. globulus* cohort survival probability, we computed a Cox Proportional Hazards Model (Cox 1972; Therneau & Grambsch 2000). Since all seedlings died during the experiment, the surviving subjects were plots with at least one living seedling. The model was produced using the *management operations* and *sowing time*, and the interaction between them, ignoring whether seedlings were derived from capsules or free seed

We assessed the effect of weather variables, management operations and their interaction on seedling mortality over the course of the experiment using Linear Mixed Models (LMM). Seedling mortality (response variable) was defined as the proportion of dead plants found at each visit relative to the number of living plants recorded in the previous visit. Explanatory variables included the *management operations* and the following weather variables, collected daily: *minimum temperature*; *average temperature*; *maximum temperature*; *precipitation*; *relative humidity*; *average wind speed*; *soil water content*; *crop evapotranspiration*. In order to minimize the influence of a reduced number of seedlings in the mortality results, we only included plots featuring five or more seedlings in the analysis. Likewise, the variable *sowing time* was not used in the analysis because there were plots that had a very low number of seedlings and others with many, leading to model overdispersal. To avoid multi-collinearity between explanatory variables, we computed variance inflation factors (VIF) to select the final set of potential predictors, using the R package *car* (Fox & Weisberg 2019). We adopted VIF>4 (Zuur et al. 2009) as the threshold for discarding correlated variables. Therefore, the following variables were not used in the model to minimise collinearity: *average temperature*, *maximum temperature, solar radiation,* and *precipitation*. We also removed from the model the variables *minimum temperature*, *relative humidity*, *wind velocity,* and the *soil water content* because they were not significant (p-value > 0.05). The LMMs was undertaken with the R package *nlme* (Pinheiro & bates 2006) using a Poisson error distribution. The variance explained by the model was obtained using the package *MuMIn* (Nakagawa & Schielzeth 2013). Comparison between competing models was performed using analysis of variance.

In order to assess the predation of *E. globulus* seeds, we compared the number of seeds predated or removed between feeding stations, feeder types and seed types using Kruskal-Wallis tests, followed by a post-hoc Nemenyi test using the R package *PMCMR* (Pohlert 2018). All statistical analyses were performed using R software (R Core Team 2019).

4.3 Results

4.3.1 Descriptive data

Among the 8,000 free seeds sown in the monitoring subplots, we recorded the emergence of 379 *E. globulus* seedlings, corresponding to a germination rate of 4.8%. Most of these seedlings emerged in the plots sown *after harrowing* (n= 10; 26%), *after control* (n= 9; 16%) and *after burn* (n=9; 15%). Of the seed sown enclosed in the 800 capsules only two *E. globulus* seedlings emerged, one in a *burn* plot (capsules shed after treatment) and the other in a *control* plot. Based on an estimate of 34,560 encapsulated seeds, the germination rate was only 0.006%. Germination occurred in 63 out of 80 plots sowed with free seeds. The number of plots of each *management operation* - *sowing time* combination r with at least one emerged seedling ranged from a minimum of 5 (in the combination *stripping – before*; i.e., seeds were sowed *before* the soil *stripping* operation) to the maximum possible of 10 (in the combination *harrowing – after*). Among the plots sown with free seeds, there was an average of 6.0 seedlings (SD = 4.7). The maximum number of emerged seedlings was 23 (23% of the seeds sown), recorded in a plot where *seeds* were sown *after* soil *harrowing*.

During the experiment, the maximum height of seedlings was 7 cm, the minimum was 2 cm, and the average modal height was 1.22 cm (se=0.12).

On the 8th of October, 13-21 days after sowing, we registered synchronized emergence across several plots (Fig. 1). In that day we recorded 149 new *E. globulus* seedlings (39.1% of all recorded seedlings). In the second monitoring, on the 16th of October, we recorded 113 new seedlings, meaning that 68.7% of all recorded seedlings emerged within the first month after sowing. Two weeks later, on the 30th of October, 96.3% of all recorded *E. globulus* seedlings had emerged. Afterward, there was a sudden drop in the number of emerged seedlings, with sporadic emergence recorded until the 14th of January, approximately 16 weeks after sowing. The average number of living seedlings in the plots started decreasing steadily at the beginning of winter (31st of December). Starting at mid-spring (May 2014), we recorded a series of mortality events, the final one at the beginning of summer (start of July). On the 21st of July 2014, approximately 10 months after sowing, and following the last major mortality event, we recorded only 30 surviving *E. globulus* seedlings (7.9% of the total germinations). At the end of the experiment, on the 17th of October 2014, nearly one year after sowing, there was only one surviving seedling, resulting from seed sown after *soil harrowing*.

4.3.2 Treatment effects on germination

The MRT used to assess the influence of *management operations*, *sowing type,* and *sowing time* on the number of emerged seedlings created five clusters of plots (Fig. 2). The most influential variable was *sowing type* (*seeds* or *capsules*), originating the first split of plot clusters, where plots sown with *capsules* (50% of all plots) had a lower average number of seedlings (0.025 seedlings per plot). The second split of the regression tree was applied to the plots sown with *seeds*, creating two groups based on the *sowing time* (*before* or *after*). Plots sown *before* the management operations (25% of all plots) had a lower average number of seedlings (2.9 seedlings) than those sown *after* (25%; 6.5 seedlings) Among the plots sown *before* the management operations, plots subject to *burning* and *stripping* (12.5% of all plots) had the lowest average number of seedlings (1.7 seedlings). Among the plots sown *after*, plots subject to *harrowing* (6% of all plots) formed a cluster with the highest average number of seedlings among all clusters (9.9 seedlings). The whole tree explained 46% of the variability in seedling emergence among plots.

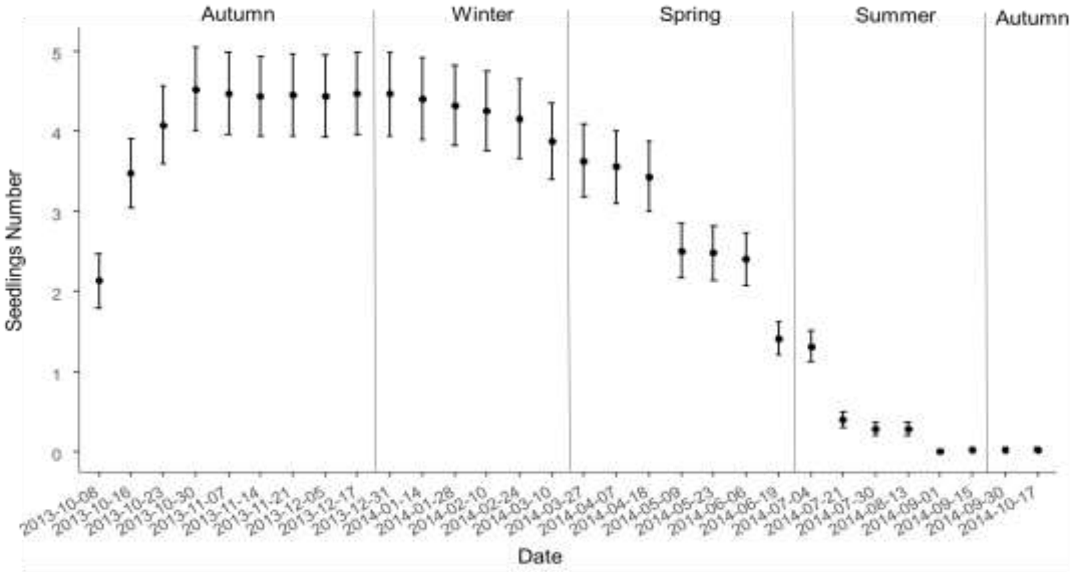

Fig. 1 - Average number of living *E. globulus* seedlings per plot in each monitoring visit. Only plots with at least one emerged seedling were considered. The error bars represent standard errors. The sowing was done between 18th and 27th of September 2013

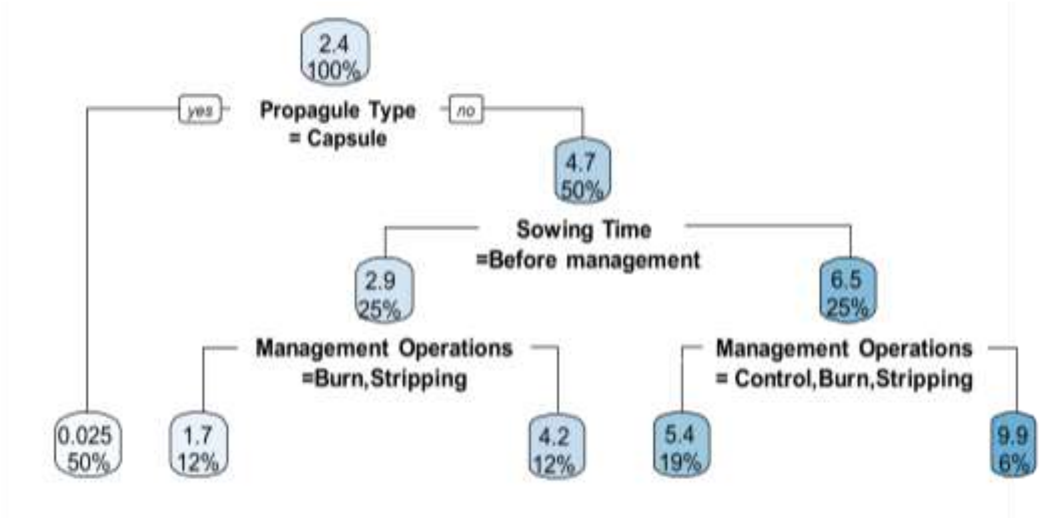

Fig. 2 - Multivariate Regression Tree with the total number of emerged seedlings in each plot, according to the *management operations* (*harrowing*; *stripping*; *burning*; *control*), the *sowing type* (*capsules*; *seeds*) and the *sowing time* (*before* or *after* the management operations). A box represents each splitting node with the treatment level(s) indicated representing the split to the left and remaining levels grouped in the right-hand split. Within each block, the average number of seedlings per plot (top number) and the percentage of the 160 plots surveyed included in that splitting node (bottom), are indicated. The colour darkness in each box is proportional to the fitted value. The length of each branch is proportional to the difference in deviance between each node and the sum of the nodes below

A GLMM was performed to assess the influence of management operations, sowing time, and their interaction on the number of germinations, considering only plots with at least one seedling established (n plots = 65). The model retained two variables, with contrasting effects. The most influential factor was soil harrowing, which positively influenced the number of emerged seedlings. The interaction of burning with seeds sown before had a negative influence on the number of germinations. The model explained 36 % of the variance (Table 1).

Table 1 - Generalized Linear Mixed Model assessing the influence of *management operations* (*harrowing*; *stripping*; *burning*; *control*), *sowing time* (*before*; *after*) and their interaction on the number of germinations (response variable). The analysis was restricted to plots registering at least one germination (n = 65)

	Estimate	Std. Error	z value	*p*-value
(Intercept)	1.795	0.166	10.819	*******
(*After management*)				
Before management	-0.138	0.195	-0.709	ns
(*Control*)				
Burn	-0.128	0.186	-0.685	ns
Harrowing	0.450	0.164	2.737	**
Stripping	-0.157	0.195	-0.805	ns
(*Control*After*)				
Burning*Before	-0.691	0.338	-2.044	*
Harrowing*Before	-0.338	0.277	-1.221	ns
Stripping*Before	-0.317	0.346	-0.918	ns

The reference level of the categorical variable is shown in brackets

Significance *p*-value: ≤ 0.001 = ***; ≤ 0.01 = **; ≤ 0.05 = *;> 0.05 = ns

4.3.3 Survival analysis and drivers of mortality

Analysing the Cox Proportional Hazards Model (Table 2), we observed that the risk of seedlings death was lower in the *harrow* plots compared with *control* plots (coef= -1.36; p= 0.009). On the other hand, plots sown *before* the management operations, had a higher risk of seedlings' death, compared with plots sown *after* management operations (coef=1.07; p=0.04).

Table 2 - Cox Proportional Hazards Model to assess the influence of each treatment on the number of deaths between two consecutive field visits. Explanatory variables were *management operations*, *sowing time,* and the interaction between these two variables

	Estimate	Std.. Error	z value	*p*-value
(Control)				
Burn	-0.393	0.477	-0.824	ns
Harrowing	-1.358	0.527	-2.578	**
Stripping	-0.915	0.504	-1.815	ns
(After management)				
Before management	1.071	0.509	2.103	*
*(Control*After)*				
Burn*Before	-1.223	0.714	-1.713	ns
Harrow*Before	0.918	0.746	1.231	ns
Stripping*Before	0.235	0.757	0.311	ns

The reference level of the categorical variable is shown in brackets

Significance *p*-value: ≤ 0.001 = ***; ≤ 0.01 = **; ≤ 0.05 = *;> 0.05 = ns

The LMM developed to assess the influence of treatments and weather on seedling mortality retained the *crop evapotranspiration* (ETc; p<0.001) and the ETc interaction with *management operations* (Table 3). In the *control* plots, the mortality due to evapotranspiration was higher than in the *burn* and *stripping* plots (p-value= 0.02 and <0.001, respectively). The model explained 42% of the variability in seedling mortality.

Given the importance of ETc for seedling mortality, we produced a plot combining the percentage of mortality in each monitoring and the ETc between this period (Fig. 4). The plot illustrates the strong positive correlation between the two variables, with higher ETc corresponding to higher mortality. July was the month that registered the highest percentage of dead seedlings.

Table 3 - Linear mixed model produced to analyse the effect of *crop evapotranspiration* (ETc), *management operations* and the interaction between these two variables on seedling mortality (number of deaths between two consecutive visits)

	Estimate	Std. Error	t-value	p-value
(Intercept)	-7.103	4.721	-1.505	ns
ETc	1.886	0.346	5.451	***
(Control)				
Burn	1.837	2.510	0.732	ns
Harrow	0.379	2.507	0.151	ns
Stripping	0.893	1.871	0.477	ns
*(ETc*Control)*				
ETc*Burn	-0.481	0.194	-2.471	*
ETc*Harrow	-0.315	0.194	-1.627	ns
ETc*Stripping	-0.563	0.148	-3.812	***

The reference level of the categorical variable is shown in brackets

Significance *p*-value: ≤ 0.001 = ***; ≤ 0.01 = **; ≤ 0.05 = *;> 0.05 = ns

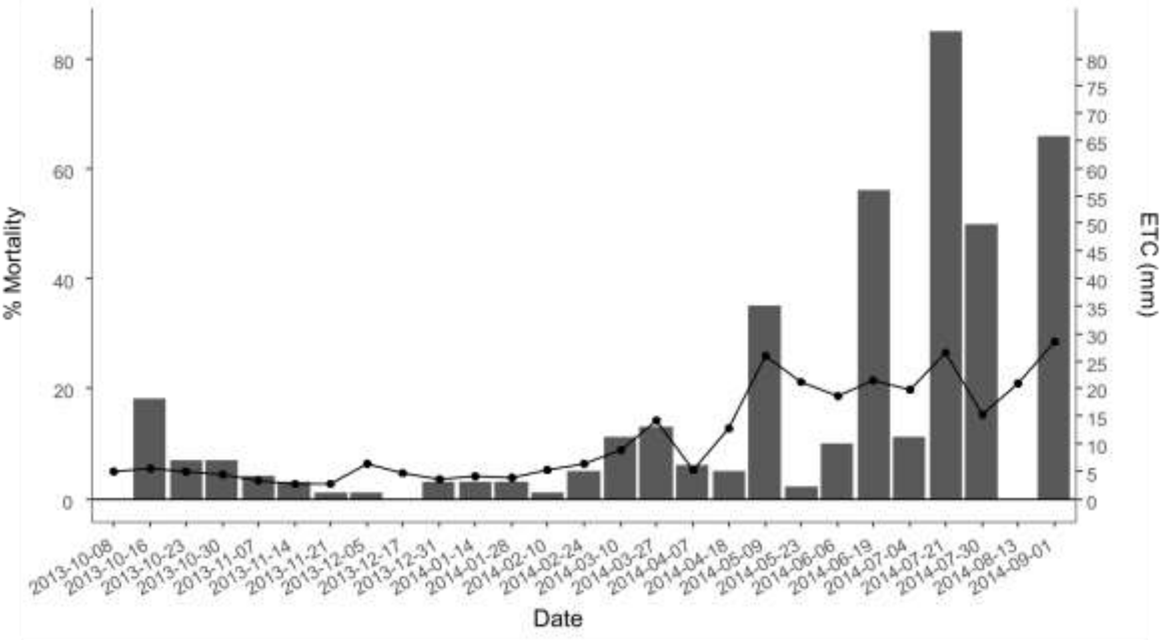

Fig. 3 - Graph showing the relationship between *crop evapotranspiration* (ETc; black line) and seedling mortality (grey bars) across time. The sowing was done between 18th and 27th of September 2013. Field surveys were performed weekly until November 2013 and twice a month afterwards

4.3.4 Seed predation

In the seed predation experiment, nearly half of *E. globulus* seeds (51.4%) were missing from the feeders (either eaten or removed) within 24 hours of exposure. Seeds from *E. globulus* were used in higher amounts than seeds from *Acacia* spp. (45.1%) and *C. salviifolius* (38.4%), even though there were no statistically significant differences regarding the daily average number of seeds used. Both vertebrates and invertebrates used all seed types, but there were no significant differences regarding the average number of seeds used between feeders with different animal exclusion treatments. In contrast, we found some spatial variability in the foraging intensity between feeding stations ($p=0.016$; Kruskal-Wallis test). One feeding station was subject to less seed predation compared to the two other feeding stations ($p < 0.05$; Nemenyi tests).

4.4 Discussion

4.4.1 The sowing experiment

Free seeds were much more successful at germinating than encapsulated seeds. Germinability tests confirmed that both seed lots had high viability. Given the number of seeds per capsule we estimated that four times more encapsulated seed was sown than free seeds. Considering that most capsules had open valves at the time of sowing, we would expect capsule sowing to be more successful. In another study, observations of seed dehiscence from capsules hanging on branches subject to scorching (to induce seed desiccation), showed that dehiscence started one week after scorching, but extended over an eight-week period, with a peak of dehiscence in the fifth week (Santos et al. 2015). Another work showed that seed dehiscence from capsules occurred over 2 to 26 days (mean 7.9 days) and was influenced mostly by desiccation rather than heating (Silva et al. 2016). In northwest Spain, Calviño-Cancela and Rubido-Bará (2013) found that only a small fraction of *E. globulus* capsules on the ground were empty, while the bulk were filled, and suggested that seeds were mainly dispersed within capsules. In our study, perhaps the time of capsule drying was not sufficient to allow the full release of the encapsulated seeds, the impact on the soil was not enough to propel seeds out of capsules, or capsules landed and remained upright and thus seed remained in the capsules and were not exposed to conditions allow germination. Another possible explanation is that the release of seeds from capsules in high densities or in heaps (Free 1951), makes them more susceptible to predation due to easier detection (Andersen & Ashton 1985; Deus et al. 2018; Hulme 1994).

Seeds of *E. globulus* do not exhibit dormancy, meaning that seeds will readily germinate when requirements are met. Under controlled environment (laboratory), most *E. globulus* seeds germinate within a few days given suitable conditions, especially moisture availability, since the temperature range is relatively wide (López et al. 2000; Reyes & Casal 2001; Silva et al. 2016). In natural conditions, germination of *E. globulus* seeds may be delayed until temperature and water requirements are met. For instance, in a semiarid region in Chile, germination of *E. globulus* seeds in a sowing experiment took more than two months (Becerra & Bustamante 2011). In northwest Spain, most germination from seeds sown in all four seasons occurred in the first two months after sowing, and practically no germinations were recorded after four months. In our study, we recorded the start of synchronized germination at 2-3 weeks after sowing in all eight treatments sown with free seed. In less than 1.5 months, practically all recorded seedlings (96.3%) had emerged. The relatively fast germination and also the germination rate may be explained by the occurrence of rain the day after the last sowing (the *after* management sowing), combined with moderate temperatures.

The *E. globulus* sowing experiment resulted in a higher seedling establishment (4.8 % of the total seeds) compared to the average of 1 to 3%, depending on habitat and less than 2.5% found by Calviño-Cancela and Rubido-Bará (2013) and (Calviño-Cancela et al. 2018) respectively. Becerra and Bustamante (2011) reported slightly higher establishment rates in open spaces, averaging 6%. In our case, seeds were dropped, simulating natural seed shed, while in other studies (Becerra & Bustamante 2011; Calviño-Cancela et al. 2018; Calviño-Cancela & Rubido-Bará 2013) seeds were buried at optimal depths (2 mm). Dropped seeds would be expected to have lower chances of germination due to factors such as physical damage, predation and direct exposure to sunlight. In this respect, some authors recommend light for germination (Langkamp 1987), while others report that full sunlight is detrimental for *E. globulus* emergence (González-Muñoz et al. 2011).

At the time of our experiment, it was unknown if seeds dispersed on the ground were likely to be harvested by animals. It is known that in Australia, particularly in southeast Australia, where *E. globulus* occurs naturally, dispersed eucalypt seeds are heavily predated by ants (Andersen & Ashton 1985; Ashton 1979). A preliminary experiment conducted in north western Spain suggested that *E. globulus* seeds were not predated (Calviño-Cancela

& Rubido-Bará 2013). However, we found that *E. globulus* seeds were highly attractive to animals, both vertebrates and invertebrates, in accordance with the findings of (Deus et al. 2018) in nearby areas, where seeds were predated by both ants and rodents. Similarly, to the findings of Deus et al. (2018), we found significant differences in foraging intensity between adjacent feeding stations, suggesting that such predation is spatially variable. According to Deus et al. (2018), most animal interaction with *E. globulus* seeds will likely result in seed destruction. Therefore, results from the seed predation experiment suggest that animal-seed interaction could have influenced the performance of the sowing experiment, and may help to explain small-scale spatial variability of seedling establishment among replications. We should note, however, that the seed predation experiment was conducted in January 2014, under lower winter temperatures, a few months after sowing in late summer (September 2013). Higher summer temperatures in September probably coincided with higher animal activity, and probably higher seed predation as observed in the Australian summer (Andersen & Ashton 1985; Ashton 1979). In contrast, we found no evidence of predation of seedlings themselves by herbivores in our experiment, even though the European rabbit (*Oryctolagus cuniculus*), which occurs in Portugal, was found to browse *E. globulus* in Chile (Becerra & Bustamante 2008).

4.4.2 Seedling germination

All management treatments had a negative impact on emergence from free seeds distributed prior to treatment, especially when plots were subject to *stripping* or *burning*. Management operations such as soil *harrowing* may have buried many seeds at unfavourable depths for germination. The relatively small size of eucalypt seeds means that if seeds are buried just a few centimetres, seedlings will not be able to emerge (Free 1951). The use of a levelling blade (*stripping*) on previously sowed plots may have also buried the seeds or perhaps dragged them outside the survey plots, even though no seedlings were found in periodic assessments outside the subplots sowed. Our results suggest that fire has a destructive effect on previously shed seeds. A study conducted in burnt *E. globulus* plantations in Portugal confirmed that wildfires destroy most previously dehisced seeds and encapsulated seeds (Santos et al. 2015). In our study, the experimental burn was conducted in herbaceous fuels with relatively low fuel loads, resulting in low fire intensity. Nevertheless, *E. globulus* free seeds do not have any particular protection against high temperatures

(Silva et al. 2016), meaning that they may be destroyed by even a small fire. However, the effect of fire on buried *E. globulus* seeds has been poorly explored (Santos et al. 2015; Stoneman 1994). Seeds shed *after management operations* were not subject to subsequent disturbance. However, seeds fell on different physical and chemical environments dependent upon the operation treatment. Soil *harrowing* created an irregular micro-topography while soil *stripping* with a levelling blade created a compacted, flat surface. In *harrowed* plots, where the best emergence occurred, *seeds*, probably found better conditions to become naturally embedded in the soil after the first rain, with plenty of microsites able to retain moisture more efficiently. Also, the irregular micro-topography of *harrowed* plots may have provided the best refuges from seed predation by vertebrates and invertebrate animals (Deus et al. 2018). Our results also confirmed that burnt areas are more favourable to seedling recruitment than the control. Calviño-Cancela et al. (2018) compared burned and non-burned areas in native forests and the number of seedlings found in the burned areas was 42.5 times higher compared to non-burned areas. Fires add chemical and physical effects that may increase the germination and seedling emergence (Fenner 2000). Fire removes competition and facilitates seed germination because of increased light reaching the soil (Florence 2004; Keeley & Fotheringham 2000). In terms of chemical effects, Águas et al. (2018) found that germination and root growth of *E. globulus* were positively correlated with the organic carbon found in burned litter from other species. Other factors can influence seed germination, like soil management intensity and the natural vegetation present in the area. These factors may explain some differences among studies assessing eucalypt invasiveness. For example, a sowing experiment in Brazil showed a low germination rate using seeds from several eucalypt species and their hybrids; in this experiment most germinations occurred in (artificially) disturbed areas (da Silva et al. 2016; da Silva et al. 2011). Another study in the USA only found *Eucalyptus amplifolia* wildlings in unmanaged areas, and no wildlings were found in intensively managed areas (Lorentz & Minogue 2015). In both studies the low germination rate was explained by higher ‘ecological resistance’ (Elton 1958) due to higher species diversity (Emer & Fonseca 2011; Lorentz & Minogue 2015).

4.4.3 Seedling survival/mortality

According to previous studies, the sowing season does not seem to influence germination, but the same does not apply to seedling survival (Fernandes et al. 2017; Rodríguez-García et al. 2011a; Rodríguez-García et al. 2011b). Fernandes et al. (2017) found survival differences between spring sowing and autumn sowing, but after the first summer, this difference was not significant, meaning that after a specific plant size, the survivors could withstand drought. Summer drought is a limiting factor for seedling survival in Mediterranean ecosystems and corresponded to the highest mortality in our experiment. It is likely that poor water and nutrient supply in a very exposed site could have facilitated the “summer effect”. Additionally, the very small plant size attained at the beginning of summer was probably a critical factor that dictated the fate of the seedlings for the rest of the experiment.

The type of treatment also had a significant effect on seedling survival. Treatments can be good for seed germination but not necessarily favourable for seedling establishment and survival (Cargill et al. 2019). The *harrowing* treatment resulted in the highest seedling survival. The existence of shelter surrounding the seedlings and a high soil and air moisture retention provided by the small soil depressions resulting from the *harrowing* operation, may have contributed to a higher survival probability (Bailey et al. 2012; Collins & Good 1987). In a study in Brazil, air humidity under 80% increased seedling mortality, while above 85%, no mortality was registered (da Silva et al. 2016). The type of *harrowing* was superficial only affecting the shallow soil layer. In case of deep *harrowing* (e.g. 80 cm deep) deeper soil horizons are also disturbed and the organic matter is more mineralized, affecting seedling survival (Madeira et al. 1989). Also, in the *control*, vegetation was left intact, which may have caused a stronger competition for light, water, and nutrients, and adversely affecting seedling survival (Kirkpatrick 1977; Stoneman 1994). These results are consistent with those of Deus et al. (2019) who studied the presence and abundance of wild *E. glob*ulus seedlings along plantation edges and found a negative influence of the herbaceous cover on the number of *E. globulus* recruits. The survival probability in the *burning* and *striping* plots, and in plots that were sown *before management operations* could have been influenced by the fewer number of seedlings in those plots.

Many abiotic conditions can influence seedling mortality across time, including water deficits, high soil-surface temperatures, low light intensity, frost, freezing temperatures, and snow (Stoneman 1994). In our experiment, the main factor contributing to seedlings mortality was *crop evapotranspiration*. Other studies performing sowing experiments registered high mortality of young *E. globulus* seedlings associated with high temperatures and drought (Calviño-Cancela & Rubido-Bará 2013; Fernandes et al. 2017). Surprisingly, the estimated soil water content was less relevant than evapotranspiration. We presume that the other weather variables that are involved in the estimation of evapotranspiration according to the Penman-Monteith equation, including solar radiation wind and temperature, may have played a role in this result. In fact, the tender leaves and stems of the *E. globulus* seedlings may have suffered from direct solar incidence and high temperature, which may have increased the mortality rate, as also suggested by Calviño-Cancela and Rubido-Bará (2013). As to the interaction with management operations, the *burn* and *stripping* treatments had a negative influence on mortality compared to the *control*. Despite the possible existence of mechanisms that could have favoured a higher mortality in the *burn* and *stripping* treatments, such as higher soil compaction in the latter (Madeira et al. 1989) and water repellence in the former (DeBano 2000; MacDonald & Huffman 2004), it seems that these effects were not as important as plant competition. In both *burn* and *stripping* treatments, the interspecific competition was temporarily removed compared with the *control* treatment. We argue that the reduced the interspecific competition for water, induced by these two treatments may explain these results, so when water availability was lower, seedlings were less adversely affected than the *control*.

4.5 Conclusions

Our results have implications for management operations that are currently applied inside and around *E. globulus* plantations. It seems that *harrowing* may be the worst way of preventing the emergence of new seedlings if the objective is to control eucalypt establishment within and around plantations. Disturbance seems to favour the initial stages of eucalypt recruitment and keeping ground vegetation undisturbed may be the best way of preventing eucalypt establishment and favouring the mortality of established seedlings. Doing *management operations after* the most favourable period of seed dehiscence also seems to be a beneficial strategy to prevent seedling emergence and survival. These effects should be assessed at the end of summer in *burn* and *stripping* areas, after the peak in mortality that occurs because of higher evapotranspiration.

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Chapter 5: Are post-dispersed seeds of *Eucalyptus globulus* predated in the introduced range? Evidence from an experiment in Portugal

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Author contribution:

a) helped conceiving the experimental design; b) helped performing field work; c) performed some statistical analyses; d) led paper writing, submission and revision.

Additional information:

The online supplementary material (OSM) associated to this work can be found in the links below:

OSM#1: https://www.dropbox.com/s/ymwftc3ohkv53hk/SupplementaryMaterial%231.pdf?dl=0

OSM#2: https://www.dropbox.com/s/95va38h9nqr302t/SupplementaryMaterial%232.pdf?dl=0

OSM#3: https://www.dropbox.com/s/fmbpood9yav063h/SupplementaryMaterial%233.wmv?dl=0

5.1 Introduction

Eucalyptus globulus Labill. is an evergreen tree native to southern Victoria (Australia), Tasmania and the Bass Strait Islands (Jordan et al. 1994). Plantations of *E. globulus* have expanded rapidly worldwide since the mid-20th century, becoming one of the most popular exotic plantation trees (Potts 2004; Rejmánek & Richardson 2011). On the other hand, *E. globulus* is also considered invasive in different world regions (Rejmánek & Richardson 2013). In Portugal, where *E. globulus* plantations are widespread, it was found that the recruitment of *E. globulus* seedlings is several times higher than in the native range (Águas et al. 2017; Catry et al. 2015; Deus et al. 2016). There has been a comprehensive attempt to understand the prolific recruitment of *E. globulus* in the introduced range (e.g. Águas et al. 2014; Becerra & Bustamante 2008; Calviño-Cancela & Rubido-Bará 2013; Catry et al. 2015; Fernandes et al. 2017), but the impact of predators on post-dispersed seeds has been poorly studied.

According to the *enemy release hypothesis*, the invasiveness of some alien species may be explained by the scarcity of natural enemies in the introduced range, such as pathogens, parasites and predators (Colautti et al. 2004; Keane & Crawley 2002; Mitchell & Power 2003). A high propagule pressure originating from plantations (Rejmánek et al. 2005; Rouget & Richardson 2003), associated with few seed losses by post-dispersal predation, may help to explain the higher recruitment of *E. globulus* in the introduced range. Seed-harvesting animals can play a key role in the dynamics of plant populations (Chambers & MacMahon 1994; Fenner & Thompson 2005; Hulme 1998). There are reports of seed-harvesting animals facilitating plant invasions through seed dispersal (Gosper et al. 2005; Holmes 1990; Richardson et al. 2000), as well as alien plants benefiting from reduced seed predation (Fenner & Lee 2001; Wolfe 2002).

In Southeast Australia, where *E. globulus* is native, seed-harvesting ants predate heavily the post-dispersed seeds from eucalypts, especially in the summer (Andersen & Ashton 1985; Ashton 1979), severely limiting eucalypt recruitment (Andersen 1982; Drake 1981; Wellington & Noble 1985). In the native range, eucalypt recruitment is strongly enhanced after wildfires, following a massive, synchronized fire-triggered seed dehiscence, that satiates seed-harvesting ants and provides a post-fire seed bed free from competition (Andersen 1989; Ashton 1979; Janzen 1969; O'Dowd & Gill 1984). To our knowledge, there is no experimental evidence of predation of post-dispersed eucalypt seeds in the introduced range. In NW Spain, in a preliminary experiment, Calviño-Cancela and Rubido-Bará (2013) did not find any evidence of predation of post-dispersed *E. globulus* seeds. There are only anecdotal reports of animals predating encapsulated eucalypt seeds outside the native range, particularly birds (Bean & Russo 1986; Calviño-Cancela 2013) and squirrels (Jacobs 1979).

In order to investigate seed predation, it is common practice to use *seed removal experiments* (e.g. Holmes 1990; Montesinos et al. 2012; Wandrag et al. 2013). In these experiments, a known amount of seeds are made available to animals, and the number of seeds removed is registered systematically. Many studies distinguish the type of animals, particularly vertebrates and invertebrates, using animal-exclusion treatments (e.g. Alba-Lynn & Henk 2010; Auld & Denham 1999), because vertebrates and invertebrates may use different foraging areas (Alba-Lynn & Henk 2010; Hulme 1997), and the fate of the seeds may depend on the type of harvesting animals (Alba-Lynn & Henk 2010; Fedriani et al. 2004; Holmes 1990). Some studies also expose the studied seeds to different habitats and micro-habitats, targeting different animal populations that may have different foraging habits and food preferences (Barberá et al. 2006; Meiss et al. 2010; Ordóñez & Retana 2004).

The present study is based on a *seed removal experiment*, conducted in Central Portugal, designed to answer a core research question related to the invasiveness of a widespread timber species: do animals play a relevant role in the fate of post-dispersed *E. globulus* seeds in the introduced range? Seeds from two other locally common species, *Acacia dealbat*a Link (alien, larger, elaiosome-bearing seeds) and *Cistus salviifolius* L. (native, similar-sized seeds), were included in the experiment for comparison purposes. The specific objectives of this experiment were: a) to investigate the occurrence of predation of *E. globulus* seeds; b) to explore spatial variation throughout the study area regarding the activity of different types of seed predators and seed preferences; c) to compare the relative importance of vertebrates and invertebrates as seed predators and their preferences between the three seed species. We had the following expectations: *E. globulus* seeds will largely escape predation, in contrast to the native range; some locations will be more intensely foraged than others, either by invertebrates or by vertebrates; *E. globulus* seeds will be the least preferred seeds, for being exotic, unlike *C. salviifolius*, and for lacking an ant-attractive elaiosome, unlike *A. dealbata* seeds.

5.2 Materials and methods

5.2.1 Study species

Eucalyptus globulus (Myrtaceae) is an evergreen tree that grows up to 55 m tall. Seeds are enclosed in woody pseudo-capsules, with narrow valves that open to release the seeds following capsule desiccation (Silva et al. 2016; Suitor 2008). Seed shed occurs throughout the year, but a massive seed shed is triggered by wildfires (Cremer 1965; Santos et al. 2015). Seeds are very small (maximum width ranging from c. 1 to 2 mm), non-dormant, short-lived and do not form a durable soil seed bank (López et al. 2000; Santos et al. 2015).

Acacia dealbata (Fabaceae) is an evergreen tree, up to 15 m, native to Australia and eastern Tasmania, regarded as an aggressive invader in Portugal and elsewhere (Lorenzo et al. 2010; Rejmánek & Richardson 2013). The fruits are pods which mature annually and release their seeds from June to August (in the Northern Hemisphere). Seeds are c. 4-5x2.5 mm, hard-coated, dormant, forming persistent soil seed banks and possess a lipid-rich elaiosome, nearly half the size of the seed.

Cistus salviifolius (Cistaceae) is a shrub, up to 90 cm, occurring spontaneously throughout the Mediterranean Basin (Moreira et al. 2012). The fruits are capsules, generally mature annually in early summer, and may take several months until all seeds are released (Bastida & Talavera 2002). Seeds are numerous, very small (c. 1 mm), hard-coated, have an endosperm and are dormant, accumulating in persistent soil seed banks (Bastida & Talavera 2002; Thanos & Georghiou 1988).

Seeds from *A. dealbata* and *C. salviifolius* were included in the experiment for the following reasons: both species occur in the study area; elaiosome-bearing seeds of *A. dealbata* are harvested by ants in this region (Montesinos et al. 2012) and should confirm the presence of seed-harvesting animals for being highly attractive, particularly to ants (Brew et al. 1989; Hughes et al. 1994; Wandrag et al. 2013); seeds from *C. salviifolius* have a similar size to *E. globulus* and are also known to be harvested by native ants (Bastida et al. 2009).

5.2.2 Study area

The experiment was established in a peri-urban area of Coimbra, in the Central West of Portugal (40º12'25'' N; 8º27'01'' W), including the campus of the Coimbra Agriculture School (ESAC) and a nearby area (map in Supplementary material #1; Fig. S1). The climate is Mediterranean with Atlantic influence, with mean annual precipitation of 922 mm and mean monthly temperatures between 21.9ºC in July/August and 9.9ºC in January (period 1981-2010; IPMA 2014). Altitude ranges between 30 and 90 m. It comprises a mosaic of land uses, including agricultural land and forest, with scattered roads, tracks and buildings. Sampling units were selected in study sites with distinct structure and composition of the vegetation. We did not aim to test habitat effect (no replications for habitat), but simply to expose the study seed species, especially *E. globulus* seeds, to potentially distinct fauna. Since local fauna should be more adapted to use seeds of local plant species as a food resource, we chose three sites, each one dominated by one of the three plant species under study.

Site characterization was made through a visual estimate of different variables within a five meters radius from the centroid of each sampling unit (ten in each site). In each of these areas, we identified all plant species with a cover greater than 5% (including canopy cover). The first site was an *E. globulus* plantation, with a total area of 0.76 ha, an average tree height of 13.8 m (SD=3.2) and an average canopy cover of 62% (SD=11.2). Besides eucalypts, measuring around 15 m height, there were a few trees of other species (*Quercus suber* L.; *Quercus robur* L.; Fraxinus angustifolia Vahl). The *E. globulu*s plantation had a shrub cover of 57.5% (SD=14.9%) and featured the highest species richness, including *Q. suber*, *Q. robur*, *Q. x coutinhoi*, *Vinca difformis* Pourr, *Ulmus* sp., *Rubus* sp., *Hedera helix* L., among others (ordered by decreasing importance). The second and the largest site (1.9 ha) was a native oak stand, with an average tree height of 6.9 m (SD=3.4) and featuring the lowest canopy cover (41.0%; SD=26.9). Most frequent trees were *Q. suber*, *Q. robur* and *Arbutus unedo* L.. The oak stand featured the highest shrub cover (63.5%; SD=20.6), even though this rate must be inflated due to the presence of *C. salviifolius* thickets in the sampling units. The third site was a small area (0.32 ha) invaded by *A. dealbata*, featuring the highest canopy cover (86.9%; SD=7.5; *A. dealbata* exclusively) and the lowest shrub cover (31%; SD=10.4), mostly composed of *A. dealbata* recruits and a few, sparse *Rubus* sp.

5.2.3 Experimental design and data collection

Five seeds of each study species, totaling 15 seeds, were placed inside *feeders* - plastic Petri dishes with no lid (10 cm diameter and one cm deep). We designed three types of feeders in order to select the type of animals that had access to the seeds. The *invertebrate feeder* was designed to prevent the access to the seeds by vertebrate animals. It was a Petri dish, placed on the ground, surrounded by a wire mesh cage (mesh size 1 cm^2). The *vertebrate feeder* was designed to prevent access by invertebrates, particularly ants and other crawling insects. This feeder was a Petri dish elevated nearly 3 cm above the soil surface, using one plastic tripod fixed in three plastic screw anchors piercing the ground, and the bottom of the Petri/tripod coated with a sticky substance (Tanglefoot®). A third type of feeder was included to assess the combined influence of both vertebrates and invertebrates, referred to as *total access feeder*. The *total access feeder* was a Petri dish placed on the ground, fixed by three nails, with unrestricted access to the seeds. In order to *control* the influence of external factors, such as seed fall from neighboring plants or seed removal by wind or rain, we included a *control feeder*, designed to prevent any access of seed-harvesting animals to seeds. Each *control feeder* was made of a *vertebrate feeder* surrounded by a wire mesh cage (scheme in Supplementary Material #1; Fig. S2). A set of these four feeder types will be referred to as a *feeding station,* each one corresponding to a sampling unit. Thirty feeding stations were evenly distributed throughout the three study sites (ten feeding stations per site), totaling 120 feeders (30 feeders of each type). Each feeding station was established adjacent to one reproductive individual of the studied species: around the stem of *E. globulus* and *A. dealbata*, or adjacent to the canopy of *C. salviifolius* thickets, with a minimum distance of 1 m between feeders (scheme in Supplementary Material #1; Fig. S3). The minimum distance to the nearest feeding stations was ten meters, in order to minimize the influence (animal and seed rain) between neighboring feeding stations. Ten meters were considered adequate, taking into account the size of the three sites.

All feeders, including the *control feeders*, were emptied every day and replenished between 9-11 a.m. with another set of five seeds of each of the studied species. Overall, each day, 600 seeds of each seed species were placed in the feeders (five seeds * 4 feeder types * 30 feeding stations), totaling 5,400 seeds during the nine days of the experiment. Feeders were monitored daily for 10 consecutive days in July 2014. Due to rain on the tenth day that caused the loss of most seeds from the *control feeders*, the results from this day were discarded. The duration of the experiment was standard for this kind of assessment, following criteria adopted by other studies (e.g. Alba-Lynn & Henk 2010; Andersen 1982; Harris & Standish 2008; Hulme 1997; Montesinos et al. 2012), and was considered adequate given the season and the type of animal agents expected to harvest the seeds. Each day, the following data was recorded for each feeder and seed species: a) number of seeds removed (coded as *missing*; seeds harvested/transported or eaten leaving no visible evidence; unknown seed fate); b) number of seeds eaten on-site (coded as *eaten*; confirmed by visible remains of the seeds in the feeder); c) number of *A. dealbata* seeds missing the elaiosome (coded as *elaiosome*; elaiosome detached or consumed; the seed remains apparently viable in the feeder).

Fresh seeds were used every day in order to assure that all seeds had equal chances of being removed by animals. Seeds were selected randomly, with no size criteria. All *A. dealbata* seeds had an attached elaiosome. Seeds were handled individually, using tweezers to place them in the feeders. A quick visual inspection on the colour and integrity of the seed, and a small pressure applied with the tweezers, were enough to ensure that seeds were apparently viable. We discarded all seeds that were soft or hollow, discoloured or apparently damaged. *Eucalyptus globulus* seeds were provided by Altri, a pulp company, and collected in September 2013 from a seed orchard. Seeds were stored in cool conditions until the beginning of the experiment. *Acacia dealbata* seeds were collected in nearby areas one year before the experiment and stored at room temperature. *Cistus salviifolius* seeds were collected in the study area in November 2013 and stored at room temperature.

5.2.4 Animal identification

A few days after the seed removal experiment, under similar meteorological conditions, we tried to identify some of the animal species using the seeds. This task was done afterwards to avoid further disturbance of the feeding stations during the experiment. In order to identify invertebrates, particularly ants, known to be the main seed-harvesters of the studied species, we placed one *total access feeder* with five of each seed species at eight locations showing the highest activity by invertebrates: four locations in the *A. dealbata* stand, three in the *E. globulus* plantation and one in the oak stand. In two consecutive days, feeders were monitored regularly between 9 a.m. and 6 p.m. by two teams searching for activity inside the feeders. Once activity was detected, the feeder was replenished with the original amount of seeds and monitored for 30 minutes. The ants that were found using the seeds were collected for identification and the type of action over the seeds (missing; eaten; elaiosome) was registered for each ant collected and for each seed species. In the locations where ants were active but not using the seeds, we collected for identification the ants crossing the feeder, apparently showing no interest in the seeds.

In our attempt to identify vertebrates, we focused on rodents, because we had found frequent evidence of rodent activity inside or next to the feeders (faeces; seed remains resulting from nibbling). In order to obtain proof of rodents using the seeds we used camera traps. Camera traps were placed only in the *E. globulus* plantation, where they were more concealed in order to prevent damage or stealing. We chose the six locations in the *E. globulus* plantation where most activity by vertebrates was registered. In each location, we established three vertebrate feeders, each containing five seeds from one of the three studied species. In this way, the footages allowed to show the action of animals with each seed species individually. In each location, camera traps were left for one day, from 5 p.m. to 10 a.m. This period was chosen for the safety of the equipment and allowed covering a few hours of daylight in the late afternoon and morning, and covering the whole night, although this meant that other vertebrate animals may have escaped identification.

5.2.5 Data analysis

In order to explore the spatial variation throughout the study area regarding the activity of different seed predators and seed preferences, we performed a Principal Component Analysis (PCA). The values of the PCA matrix were the proportions of used seeds (*missing*; *eaten*; *elaiosome*) in relation to the total number of available seeds of each species (9 days x 5 seeds per day = 45 seeds), for each combination feeder type x seed species (3 feeders x 3 seed spp. = 9 variables). Therefore, the PCA was developed using a 30 (feeding stations) x 9 (variables) matrix. A biplot was built based on the PCA results, aiming at detecting clusters of stations sharing similar patterns of seed predator activity and preferred seeds. These patterns were inferred based on the proportions of used seeds for each combination feeder type x seed species. We built a map of the study area to assess the distribution of the feeding stations according to the clusters they belong to.

We modelled the effect of explanatory variables: feeder type (three levels: *invertebrate*, *vertebrate* and *total access feeders*), seed species (three levels: *E. globulus*, *A. dealbata* and *C. salviifolius*) and their interaction, on the proportion of used seeds (out of 45 seeds available along the nine days; n= 3 feeders x 3 seed spp. x 3 sites x 10 stations = 270) using Generalized Linear Mixed Models (GLMM) with a binomial error distribution, following the procedures described in Zuur et al. (2009). Study site (eucalypt plantation, acacia stand and oak stand) and feeding station (10 stations in each site) were included in the model as nested random effects using function glmmPQL of the R package MASS (Venables & Ripley 2002). This function fits a GLMM model with multivariate normal random effects, using a Penalized Quasi-Likelihood approach (Venables & Ripley 2002). Model fit was assessed by the marginal R^2 (variance that is explained by the fixed terms of the model), using the R package MuMIn (Bartoń 2018), according to Nakagawa and Schielzeth (2013). Post-hoc pairwise comparisons between all combinations of significant factor levels were performed through Tukey tests with adjusted p-values using the R package multcomp (Hothorn et al. 2008).

In a second stage, we used a similar procedure to model the proportion of used seeds separately for each seed species, but using feeder type, type of animal action over the seeds (missing; eaten; elaiosome-detachment) and the interaction between the two factors, as explanatory variables. Therefore, four models were produced (one general and one for each studied species) and the corresponding multiple comparisons.

An additional analysis aimed at assessing the potential of seed depletion of each seed species, by retaining only the occasions (observations) where any seed was used (missing; eaten; elaiosome-detachment), *i.e.* occasions where we know that animals found the seeds and could reveal their preferences. Therefore, for each feeder type and seed species, we computed the frequencies of occasions (animal visits) where none (0), part (1-4) and all (5) seeds were either eaten or missing. Elaiosome-detachment on *A. dealbata* seeds was not accounted as depletion, since the seeds remain apparently viable in the feeder. The maximum number of occasions (visits by animals) for each seed species in each feeder type would be 270 (30 feeders of certain type x 9 days). Using these frequencies, we produced contingency tables for *invertebrate*, *vertebrate* and *total access feeders*, and tested, for each one, the independence between seed species and intensity of seed depletion using chi-square tests. All statistical analysis were performed using R software (R Core Team 2017).

5.3 Results

5.3.1 Seed predators and seed preferences

Only 27 out of 4,050 seeds (0.7%) were missed from the *control feeders* and only two extra seeds from *C. salviifolius* were found inside a feeder. Therefore, we assumed that there was no relevant interference of external agents on seed removal. A total of 2,901 seeds, out of 12,150 available (23.9%), were used by animals (missing; eaten; elaiosome-detachment) during the experiment, including 1,177 from *E. globulus*, 1,200 from *A. dealbata* and 524 from *C. salviifolius*. The day with the lowest number of used seeds was the first day, with 101 out of 1,350 seeds (7.5%). The number of used seeds increased daily along the experiment until the seventh day, when a maximum was reached (492 seeds; 36.4%), practically stabilizing afterwards. This temporal dynamics was similar for all seed species and feeder types, but not for the three study sites, since the daily total of used seeds stabilized around the fourth day in the *E. globulus* plantation (Fig. 1).

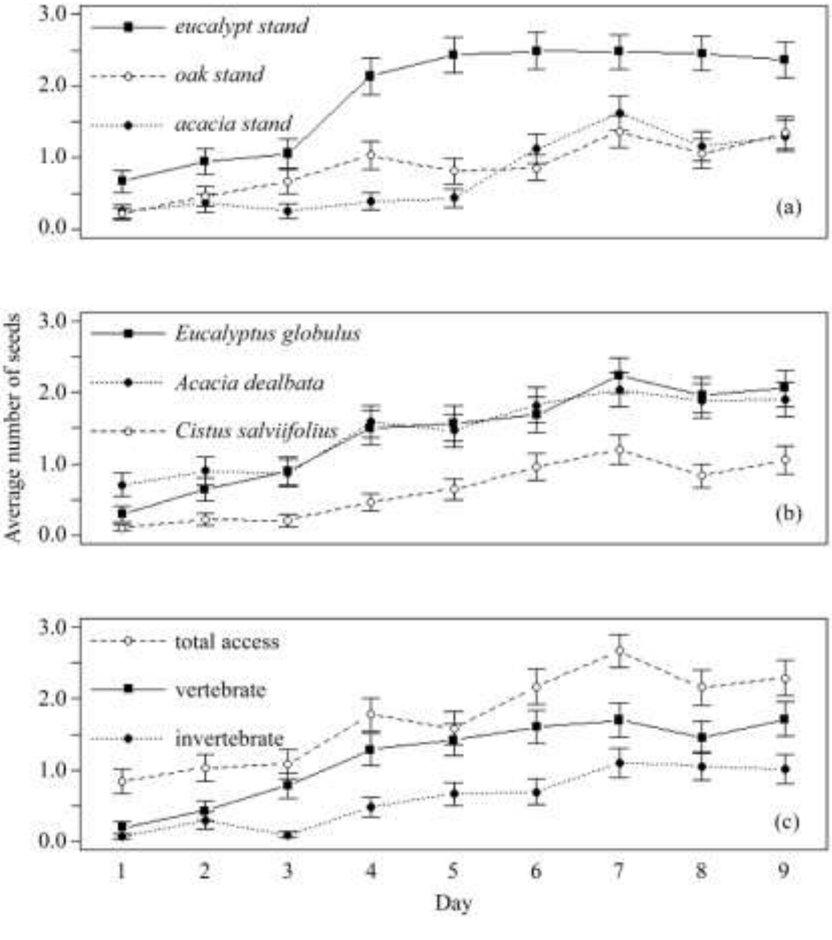

Fig. 1 - Average daily number of used seeds (missing; eaten; elaiosome-detachment) per feeder (±SE), from a total of five seeds. Data pooled for each day by (a) study site, (b) seed species and (c) feeder type

The first two components of the PCA explained 83% of the variance. The analysis of the biplot allowed distinguishing three clusters of feeding stations, characterized according to both the rate of seed use by animals and the predominant animal group (vertebrates; invertebrates), regardless of the seed species (Fig. 2). One first cluster of 13 stations (green cluster) shows areas with reduced or negligible seed predation (≤ 15% of seeds used), distributed mainly across *A. dealbata* and *C. salviifolius* stands. A second cluster (orange cluster) comprises 11 stations where vertebrate activity was predominant: eight located in *E. globulus* plantation and three in *C. salviifolius* stand. The third cluster (blue cluster) comprises six stations with mostly invertebrate activity: four in *A. dealbata* stand, one in *E. globulus* plantation and one in *C. salviifolius* stand (Fig. 3).

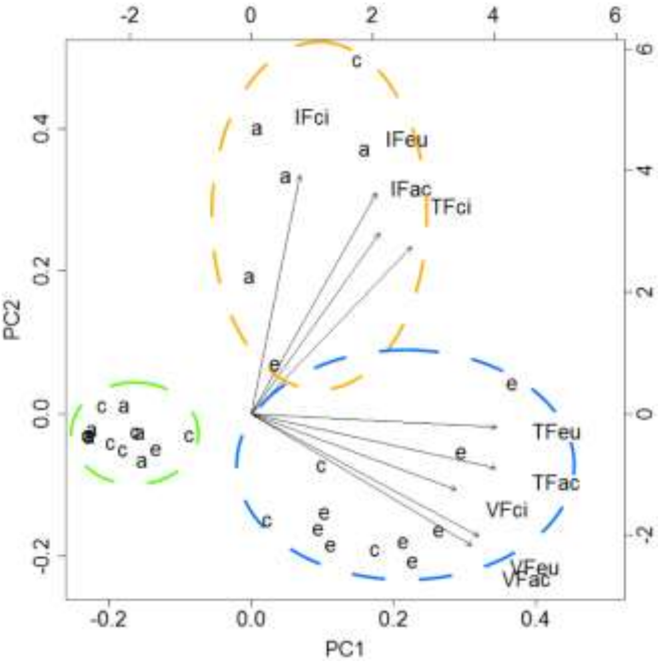

Fig. 2 - Biplot resulting from the Principal Component Analysis. Dashed circles enclose clusters of feeding stations according to the following patterns: negligible seed predation (green circle); predominance of invertebrates (orange); predominance of vertebrates (blue). Letters a, c and e, represent the feeding stations located, respectively, in the *A. dealbata* stand, the oak stand and the *E. globulus* plantations. Each group of four letters represents a variable, composed by the feeder type (IF: invertebrate; VF: vertebrate; TF: total access feeder) and the seed species (ac: *A. dealbata*; ci: *C. salviifolius*; eu: *E. globulus*)

The general GLMM explained 27% of variance. The coefficients of the fixed model terms, feeder type, seed species and their interaction, were all significant (model description in Supplementary Material #2). Pairwise comparisons showed that seeds from *E. globulus* and *A. dealbata* were used in similar proportions in the *total access feeders*, the *vertebrate feeders* and the *invertebrate feeders*. Seeds from *C. salviifolius* seeds were less used than the alien seeds in the *total access feeders* and the *vertebrate feeders* ($p<0.01$ for both comparisons), but not in the *invertebrate feeders*. Alien seeds were used in greater proportions in *vertebrate feeders* than in *invertebrate feeders* (p=0.03; p<0.01; respectively). No differences among feeders were found for *C. salviifolius* (Fig. 4).

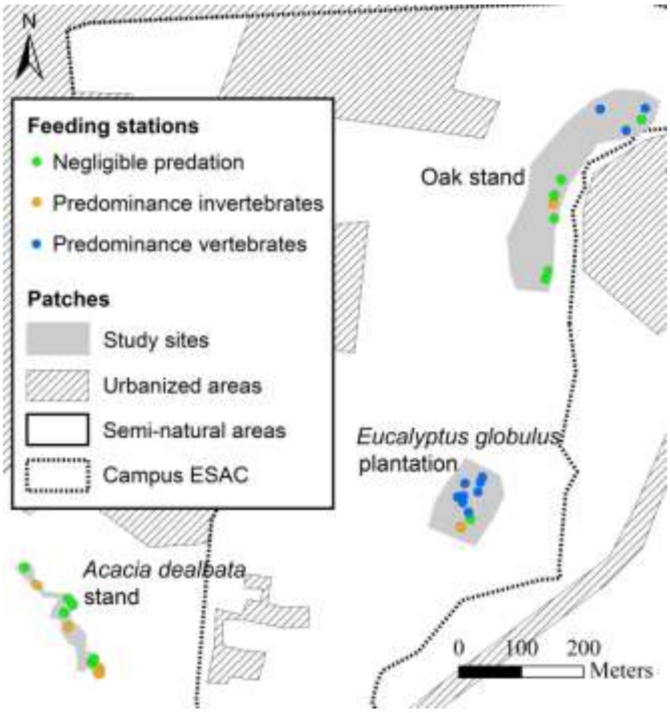

Fig. 3 - Location of the feeding stations according to shared patterns regarding the predominant seed predators and the level of seed predation

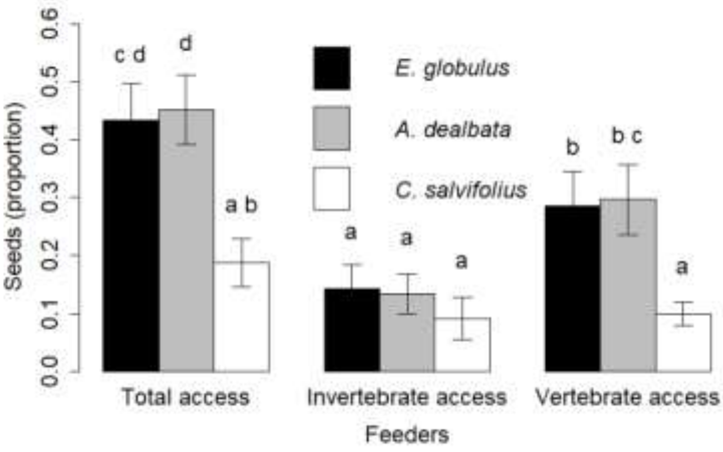

Fig. 4 - Proportion of used seeds (missing; eaten; elaiosome-detached) according to the seed species and feeder type. Different letters above the bars indicate significant differences (post-hoc pairwise Tukey tests)

The GLMM for *E. globulus* seeds explained 48% of variance with feeder type, type of action over the seed (missing; eaten; elaiosome-detachment) and the interaction between these two variables presenting significant model coefficients. The proportion of eaten seeds was significantly lower than the proportion of missing seeds in *total access feeders* and *invertebrate feeders* ($p<0.01$ for both comparisons) but not in *vertebrate feeders*. The proportion of eaten seeds was much lower in the *invertebrate feeders* than in *total access feeders* and *vertebrate feeders* ($p<0.01$ for both comparisons) (Fig. 5).

The GLMM for *A. dealbata* seeds explained 65% of variance with both variables and their interaction showing significant coefficients. The proportion of missing seeds was significantly higher in *total access feeders* and *vertebrate feeders* than in *invertebrate feeders* ($p<0.01$ for both comparisons). In *invertebrate feeders*, no differences were found between seeds missing, seeds eaten and with removed elaiosome (Fig. 5).

The GLMM for *C. salviifolius* seeds explained 74% of variance with both variables being significant, but not their interaction, which was therefore removed from the final model. The proportion of eaten seeds was significantly lower than the proportion of seeds missing in *total access feeders* and *vertebrate feeders* ($p<0.01$ for both comparisons), and was absent in in*vertebrate feeders* (Fig. 5).

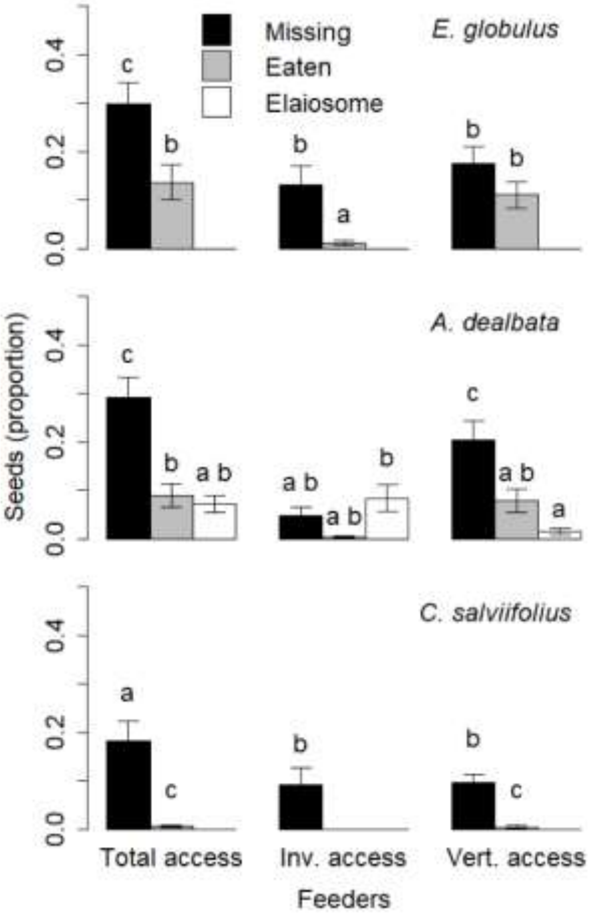

Fig. 5 - Proportion of used seeds according to the seed species, feeder type and the type of action over the seed (missing, eaten; elaiosome-detachment). Different letters above the bars indicate significant differences (post-hoc pairwise Tukey tests)

The frequencies of occasions (animal visits) where none (0), part (1-4) or all (5) seeds were eaten or missing were significantly related with the seed species (p<0.01 for all feeders). *Total access feeders* registered the greater number of occasions where any seed was used (missing; eaten; elaiosome-detachment) by animals (167 out of 270 opportunities; 61.9%). In *total access feeders*, *E. globulus* seeds had the highest proportion of occasions where all five seeds were eaten or missing (63.5%) and the lower proportion of occasions where only part of the seeds were missing or eaten (8.9%) *Invertebrate feeders* had the lower number of occasions where any seed was used (70 out of 270 times; 25.9%). In the *invertebrate feeders*, all five *A. dealbata* seeds were missing or eaten only in one occasion (1.4%), whereas *C. salviifolius* and *E. globulus* were completely depleted (missing or eaten) in more occasions (28.6% and 40.0%, respectively). Moreover, *E. globulus* seeds registered the lowest proportion of occasions with no seeds missing or eaten (35.7%). In *vertebrate feeders*, there were 105 occasions when any seed was used by animals: *C. salviifolius* seeds registered the lowest frequency of all five seeds missing or eaten (3.8%) and the highest proportion of zero seeds missing or eaten (42.9%). *Acacia dealbata* and *E. globulus* seeds had all five seeds missing or eaten in 61.9% and 68.6% of occasions, respectively (Fig. 6).

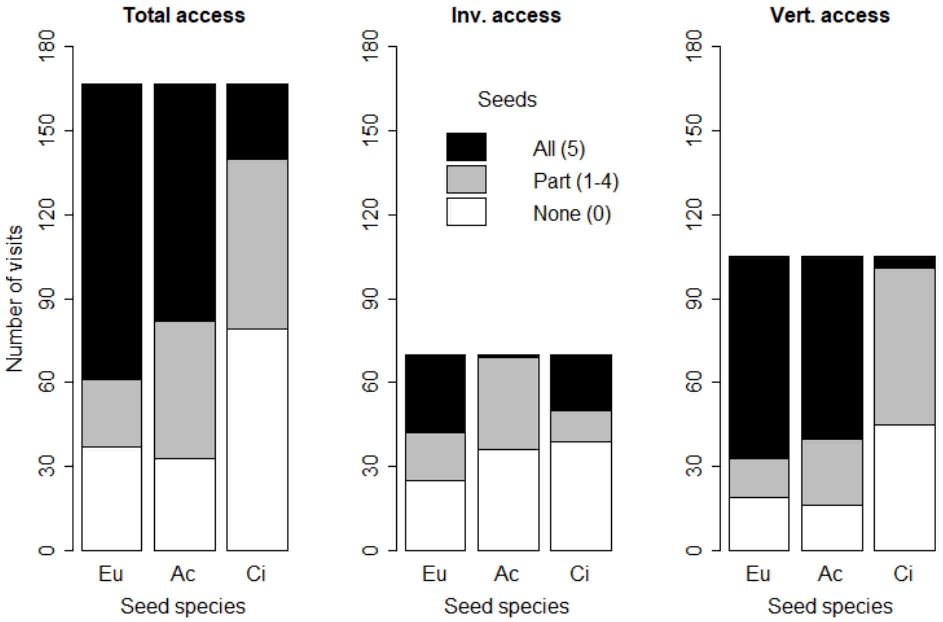

Fig. 6 - Number of animal visits (any seed used on a given day: missing; eaten; elaiosome-detached) where none (0), part (1-4) or all (5) seeds were missing or eaten, according to the feeder type and seed species. Eu: *Eucalyptus globulus*; Ac - *Acacia dealbata*; Ci: *Cistus salviifolius*

5.3.2 Identified animals

Four ant species were observed using the seeds in different locations. Individuals from *Pheidole* sp., observed in one station in the *A. dealbata* stand, transported seeds of the three species. *Tetramorium* sp., present in two locations in the *A. dealbata* stand, were observed eating (or perhaps detaching) the elaiosome of *A. dealbata* seeds, and transporting *E. globulus* and *C. salviifolius* seeds. *Leptothorax affini*s was found in one location in the *E. globulus* plantation and only showed interest in the elaiosome of *A. dealbata* seeds. *Temnothorax* sp. was observed in one location in the oak stand transporting *A. dealbata* and *E. globulus* seeds. In other locations, other ant species apparently had no interest in any of the seed species, namely *Plagiolepis pygmaea* in one location in the *E. globulus* plantation, *Linepithema humile* in two locations in the *E. globulus* plantation and *Crematogaster scutellaris* in one location in the *A. dealbata* stand.

The camera traps recorded small rodents (*Apodemus sylvaticus*) in four out of six locations within the *E. globulus* plantation. Individuals from this species apparently predated seeds of all species. The feeder containing *E. globulus* seeds was depleted in the four locations, the feeder with *A. dealbata* seeds was depleted in three locations (apparently one feeder remained unnoticed) and two feeders with *C. salviifolius* seeds were partially depleted (apparently seeds were ignored in two feeders). Seed remains (shattered testa) were consistent with observations from the main experiment. Edited footage is available in Supplementary Material #3.

5.4 Discussion

5.4.1 Seed predators and seed preferences

One of the key findings of this study is the experimental demonstration of predation (and removal) of post-dispersed *E. globulus* seeds in the introduced range, by both vertebrates and invertebrates. The hypothesis that *E. globulus* seeds largely escape predation was rejected, since nearly half the seeds were rapidly eaten or missing from *total access feeders*. The rate of seed use from *total access feeders* is a good indicator because it reflects the combined influence of vertebrates and invertebrates in the study area. The use of *E. globulus* seeds by animals, together with the other seed species, occurred in the three study sites, suggesting that local fauna rapidly start using newly arrived seeds. However, it is important to note that the other half of *E. globulus* seeds remained intact. This contrasts with reports of eucalypt seeds in the native range that found a near depletion by seed-harvesting ants (Ashton 1979; Drake et al. 2009; Wellington & Noble 1985). Nevertheless, like many seed removal experiments, the rates of seed use may be inflated and should be regarded with caution for multiple reasons: the animals may become accustomed at foraging those locations, as shown by the daily increase of used seeds; any olfactory clue from one of the seed species may have influenced the finding of other seeds; seeds displayed in clumps, and at bare sight, are more easily detected (Andersen & Ashton 1985; Hulme 1994; Myster & Pickett 1993); the experiment was conducted in the summer, when animal activity is higher.

Invertebrates, particularly ants, had a lower importance in this study, which was unexpected for several reasons. First, in the native range, it is well documented that seeds of both *Eucalyptus* (Ashton 1979; Drake 1981) and myrmecochorous *Acacia* seeds (Andersen & Ashton 1985; Auld 1986) are highly attractive to ants. However, we must take into account that in Australia, seed-eating rodents are scarce, contrasting with an exceptionally abundant and diverse ant fauna (Morton 1985), with a profusion of myrmecochory (Berg 1975; Milewski & Bond 1982). Second, in the introduced range, ants have been reported as the predominant seed harvesters of myrmecochorous *Acacia* spp., while rodents have a minor impact (Holmes 1990; Wandrag et al. 2013). Third, in the region of the present study, ant communities are ubiquitous and relatively diverse (Cammell et al. 1996), and we could only find reports of ants using both *A. dealbata* (Montesinos et al. 2012) and *C. salviifolius* seeds (Bastida et al. 2009) in nearby regions. The lower importance of ants may be explained by: the availability of alternative food sources; the disruption of foraging pathways due to the presence of other ants, such as the invasive *Linepithema humile* (Human & Gordon 1996), which was observed in some locations where seeds remained intact; the satiation by concurrent dehiscence (Holmes 1990); and the lower probability of feeder encounter due to small-scale patchiness of ant foraging (Arnan et al. 2010).

Contrary to our expectations, invertebrates, particularly ants, did not prefer *A. dealbata* seeds. We expected that the elaiosome would attract a wider array of ant species, both granivorous and non-granivorous (Clark & King 2012; Hughes et al. 1994). Likewise, the native *C. salviifolius* seeds were not preferred, compared to *E. globulus* seeds. In fact, all seed species were used by invertebrates in similar proportions. It is worth noting that *A.*

dealbata seeds were seldom depleted by invertebrates. Instead, many seeds had the elaiosome removed, a procedure normally associated with smaller ants (Auld & Denham 1999), suggesting that some ants experienced difficulties at displacing these seeds. *Acacia dealbata* seeds remain viable without the elaiosome, even though the chances of being displaced decrease (Montesinos et al. 2012; Wandrag et al. 2013). This finding contrasts with studies reporting a generalized dispersal of myrmecochorous *Acacia* seeds by ants in the introduced range (Holmes 1990; Montesinos et al. 2012; Wandrag et al. 2013). On the other hand, *E. globulus* seeds were depleted from *invertebrate feeders* on more occasions, while there were fewer occasions of *zero* seeds eaten or removed. The smaller size and weight of *E. globulus* seeds, and the soft seed testa that enables an easy meal (Andersen & Ashton 1985), may have favoured their selection in relation to the larger seeds of *A. dealbata* and the hard coated seeds of *C. salviifolius*.

In contrast, vertebrates were the main seed predators, removed a considerable amount of *E. globulus* and *A. dealbata* seeds. Footage from camera traps recorded a small native rodent species, *Apodemus sylvaticus*, removing all seed species. It is possible, however, that other vertebrates such as birds may have escaped our surveillance. Marchante et al. (2010) suggests that, in Portugal, birds (*Streptopelia decaocto*) may have ingested and successfully dispersed *A. longifolia* seeds. Birds were also observed feeding on eucalypt seeds in Spain (Calviño-Cancela 2013) and California (Bean & Russo 1986). To our knowledge, there is no previous evidence of rodents feeding on *Acacia* and *Cistus* seeds in Europe. Some factors may help to explain the predominance of vertebrates, particularly rodents, in this study. First, they are less easily satiated than invertebrates (Abramsky 1983; Hulme 1998). This was supported by the depletion of *E. globulus* and *A. dealbata* seeds in most occasions where *vertebrate feeders* were *visited*, and the obtained footage showing that a rodent can rapidly deplete all seeds. Second, small rodents may forage the same areas repeatedly by using pathways determined by prior trails (Jamon 1994), facilitating recurrent visits to same feeders; this may help to explain the association (same cluster) between *total access* and *vertebrate feeders* in the PCA biplot, particularly in the case of *E. globulus* and *A. dealbata* seeds. Third, vegetation structure and the fact that the feeders were relatively close to each other (minimum distance of 10 m), may have enhanced the performance of small rodents in this study. In fact, vertebrate activity was mostly accomplished in the *E. globulus* plantation that, unlike traditional mono-specific plantations of *E. globulus*, displayed considerable plant diversity, providing concealment and diverse food sources for small rodents (Gill & Marks 1991; Meiss et al. 2010). In contrast, vertebrate activity was unnoticed in the mono-specific, understory-free *A. dealbata* stand, and concentrated in only three neighboring stations of the oak stand, probably because rodent activity was limited by a sparse shrub cover (Benhamou 1991).

5.4.2 Seed fate and implications for *E. globulus* establishment

The obtained footages (Supplementary Material #3) and the shattered seed testa confirm that small rodents predated *E. globulus* seeds. We found no clues about the fate of most seeds missing from *vertebrate feeders*. However, to our knowledge, there is no evidence of small rodents hoarding *Eucalyptus* seeds or similar-sized seeds. There are just anecdotal reports of birds feeding on eucalypt seeds (Bean & Russo 1986; Calviño-Cancela 2013), but endozoochory of *E. globulus* seeds is unlikely due to the thin testa and seed softness. Therefore, seeds missing from vertebrate feeders were likely predated, resulting in considerable seed losses.

In the *invertebrate feeders*, a few *E. globulus* seeds were found eaten, including some left as a hollow seed, an evidence attributed to feeding by ants in Australia (Ashton 1979). Field observations suggested that different ant species, such as *Pheidole* spp., *Tetramorium* spp. and *Temnothorax* spp., may have transported *E. globulus* seeds, thus some of the missing seeds may have been displaced towards subterranean ant nests, which happens with *Eucalyptus* seeds in the native range (Ashton 1979; Wellington & Noble 1985). Non-myrmecochorous seeds, such as *E. globulus* seeds, are normally transported to the nest of granivorous ants (Bas et al. 2009; Drake 1981), thus the most probable fate is being eaten (Ashton 1979). However, successful ant-mediated dispersal may occur: *Eucalyptus* seeds may be stored by non-harvester ants (Berg 1975), occasionally at a depth suitable for germination (Andersen & Ashton 1985); depending on seed and ant traits (Gorb & Gorb 1999), some seeds may be dropped/dispersed along the way (Arnan et al. 2010; Retana et al. 2004); non-myrmecochorous seeds may be mistakenly rejected into refuse piles outside ant nests (Levey & Byrne 1993; Retana et al. 2004).

The spatial variation found in the study area regarding the predominant seed predators and the rates of seed use may imply different seed fates depending on the location where they are shed: a) predation by vertebrates; b) predation by invertebrates with occasional dispersal events; c) escape predation and integrate soil seed banks. Therefore, the spatial variation of seed predation and dispersal by animals may shape the distribution of many plant species (Alba-Lynn & Henk 2010; Andersen 1982; Hulme 1997, 1998; Whelan et al. 1991). Several factors beyond the purpose of this study may explain this spatial variation, such as habitat and micro-habitat traits (Alba-Lynn & Henk 2010; Manson & Stiles 1998; Whelan et al. 1991), predator risk for seed-eating animals (Sivy et al. 2011), season and phenology of plant species (Bastida et al. 2009), seed abundance (Holmes 1990; O'Dowd & Gill 1984) and alternative food sources. Locations with reduced or negligible seed predation may be privileged areas for seedling establishment, because seeds have greater chances to become embedded in the soil or litter, and less likely to be detected and predated in the future (Andersen & Ashton 1985; Hulme 1994; Vander Wall 1994). Seeds of *E. globulus* have no dormancy and therefore a few days may be enough for germination (López et al. 2000; Silva et al. 2016).

Our findings encourage further exploration of the role of seed-harvesting animals on the invasiveness of eucalypt species in the introduced range. Probably, throughout the introduced range, animals predate and disperse post-dispersed seeds of other widespread eucalypt species (e.g. Harwood 2011; Rejmánek & Richardson 2013), with implications for the demography and the dynamics of the populations. Further research is needed to evaluate the suitability for seedling establishment and survival of the areas where seeds largely escape predation. Also, the seasonal variation of seed predation, observed in the native range (Andersen & Ashton 1985; Ashton 1979), is likely to occur in the introduced range, and is worthy of exploration since seed dehiscence occurs throughout the year (Calviño-Cancela & Rubido-Bará 2013; Cremer 1965). It may be also appropriate to investigate the role of other animals on the fate of post-dispersed eucalypt seeds (e.g. birds). The abundance and the scattered dispersion of areas of reduced seed predation, together with site traits, may therefore help to explain the heterogeneous recruitment patterns of *E. globulus* seedlings found in previous studies (Águas et al. 2014; Águas et al. 2017; Calviño-Cancela & Rubido-Bará 2013; Catry et al. 2015; Fernandes et al. 2016; Fernandes et al. 2017).

5.5 Conclusions

Seeds of *E. globulus* were highly attractive to local fauna. Surprisingly, *E. globulus* and *A. dealbata* seeds were used in similar proportions by both vertebrates and invertebrates, despite the larger size and the elaiosome of the latter. Also, *E. globulus* seeds were more attractive to vertebrates than the native, similarly-sized seeds of *C. salviifolius*. Vertebrates were the most important seed predators, particularly of *E. globulus* and *A. dealbata* seeds. Invertebrates were less important and used all seed species in similar proportions. We found spatial variation throughout the study area regarding the predominance of different types of seed predators and the rate of seed predation: predominance of vertebrates; predominance of invertebrates; negligible seed predation. Locations with negligible seed predation were abundant and scattered across the study area, and may help to explain the heterogeneous recruitment patterns of *E. globulus* seedlings found in previous studies.

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Chapter 6: Natural establishment of eucalypts in the introduced range: a review

Author contribution:

a) performed the literature survey; b) retrieved quantitative data from studies; c) manuscript writing.

Additional information:

Unlike the previous chapters, this study was not published. This manuscript was prepared specifically for this PhD. thesis, being formatted as a standard book chapter, even though a refined version of it is planned to be submitted later to an international journal.

6.1 Introduction

Eucalypts are evergreen, hardwood plant species from the family Myrtaceae. They comprise a large, diverse group of more than 800 plant species from the genera *Eucalyptus*, *Corymbia* and *Angophora*, native to Australia and surrounding islands (Nicolle 2018). Eucalypts were probably seen for the first time by Portuguese explorers in the early 16th century in Timor. In the late 18th century, under the expeditions of Captain James Cook from the Royal Navy, the first specimens were collected and brought to Europe (Doughty 2000; Kantvilas 1996). Soon, eucalypts would become extremely popular as novel exotics, being introduced into many countries since the late 18th century (Potts et al. 2004; Silva-Pando & Pino-Pérez 2016).

Eucalypts would soon reveal very good qualities such as fast growth, ease of management through coppicing, wood quality and environmental tolerance. As stated by Navarro de Andrade (1881-1941), who first tested and investigated eucalypt plantations in Brazil, 'eucalyptus is the only tree yielding first quality wood that can be utilised by the same man who planted it' (Doughty 2000). Eucalypts started being cultivated for different purposes, such as windbreaks, drying swamps, wood and timber productions (Potts 2004). At the beginning of the 20th century, trials to produce eucalypt pulp began, and along the following decades different species revealed to be exceptional pulp sources (Doughty 2000). The increasing demand for short-fiber pulp led to a vertiginous expansion of eucalypt plantations during the 20th century in many countries (Potts 2004).

Today eucalypts are one of the most popular and important trees in many countries, being found in all continents except Antarctica. Plantations are aimed at different usages such as paper production, timber, firewood and charcoal, bioenergy, essential oils, honey production, ornamental and ecosystem restoration (Henry 2014). Around 372 eucalypt species have been introduced outside their native range (Hui et al. 2014), but the bulk of eucalypt plantations is composed by a few species, particularly *E. camaldulensis, E. grandis, E. tereticornis, E. globulus, E. nitens, E. urophylla, E. saligna, E. dunnii*, *E. pellita* and some of their hybrids, which together represent over 90% of eucalypt plantations (Stanturf et al. 2013). It is estimated that eucalypt plantations cover today more than 20 million ha worldwide (200,000 km^2) (Rejmánek & Richardson 2011).

Many eucalypt species found favourable conditions to thrive by successfully reproducing in the introduced range. In the last decades, there has been an increased concern about the invasive potential of some eucalypt species. Weed risk assessments suggest that some eucalypt species fulfil the requirements to become invasive, in countries such as USA (Gordon et al. 2012), South Africa (Nel et al. 2004), Brazil (Ziller et al. 2019), Australia (Virtue & Melland 2003), India (Jaryan et al. 2013) and Portugal (Marchante et al. 2014). National and global databases identify several eucalypt species as 'naturalised' (Pyšek et al. 2017) and a few species as 'invasive' (Haysom & Murphy 2003; Rejmánek & Richardson 2013) in different regions of the world. In some countries, large sums of money are already spent controlling eucalypt invasion (Andreu et al. 2009; van Wilgen et al. 2012).

Richardson et al. (2000b) attempted an objective definition of 'naturalised' and 'invasive' plants. *Naturalised plants* were defined as alien plants that reproduce consistently nearby parent plants, sustaining populations over many life-cycles. *Invasive plants* were defined as naturalised plants producing reproductive offspring, often in very large numbers, at considerable distances from parent plants. In that work, the process of invasion was conceptualised as a series of biotic and abiotic barriers that alien plants do (or do not) overcome, from introduction through human agency, to the invasion of disturbed or natural habitats.

The ecological impacts of eucalypts, in particular their invasive potential, have been a matter of polarised discussion and controversy in many countries, and conclusions are frequently anecdotal and highly context-dependent (Rejmánek & Richardson 2011). However, research on the natural establishment of eucalypts and its drivers has intensified in the last years, and to today we can find a large body of literature providing empirical evidences through field surveys and experiments on a variety of species across the world.

This work aims to make a comprehensive review of the studies assessing the natural establishment of eucalypts in the introduced range. This review is strongly based on a literature survey of the studies providing empirical evidences through field observations. The main objectives are: to provide an overview of the scientific literature on this topic; to assess indicators of the invasive potential of eucalypt species, including offspring abundance and dispersal distances; to synthesise the main factors influencing natural establishment. We then discuss research needs and some notes for managers. Based on our overall assessment, we share some thoughts about monitoring and management.

6.2 Eucalypt biology and ecology

Eucalypts are native to Australia and surrounding islands. Most of eucalypt species can be found around the maritime edges of Australia, where species' richness is higher (Hui et al. 2014), but eucalypts occupy a wide range of habitats and environmental conditions (Boland et al. 2006; Doughty 2000). Some species have relatively wide ranges (e.g. *E. camaldulensis*; *E. socialis*) while others are confined to very small niches (e.g. *E. retusa*; *E. boliviana*) (Hui et al. 2014).

Eucalypts encompass a variety of life-forms. Most eucalypt species are trees, some of which reaching more than 70 m tall (e.g. *E. regnans*; *E. diversicolor*; *E. grandis*; *E. deglupta*), while some species develop into shrubs (e.g. *E. moorei*; *E. vernicosa*) or 'mallees' (small, multi-stemmed trees; e.g. *E. livida*; *E. socialis*), even though there may be some variability of life-forms within the same species, caused by factors such as tree age, climate or disturbances (Williams & Brooker 1997). Eucalypts developed different regenerative strategies, which include obligate seeders, lignotuber sprouters, stem sprouters and combination sprouters (Nicolle 2006). Many eucalypt species are heteroblastic, transitioning from juvenile to adult leaves, associated with changes in shape, position, anatomy, physiology and chemistry of their leaves (Velikova et al. 2008).

The name *Eucalyptus* derives from the Greek words *eu* (well) and *kaluptos* (covered), as a reference to the operculum covering the flower bud, formed by the fusion of petals and/or sepals. The operculum is later shed under the pressure of the emerging stamens at the time of flowering. Flowering time may vary considerably between species, within species and even within the same stand (Florence 1964; House 1997). In the native range, different animals such as insects, birds, marsupials and a few bat species are the main pollinators (House 1997; Potts 2004).

Eucalypts generally produce copious quantities of flowers and fruits, many ovules and a relatively high number of seeds (House 1997). Fruits are woody capsules, featuring valves that open to release the seeds following capsule desiccation (Cremer 1965b; Silva et al. 2016). The number of seeds per capsules varies between species, individuals and seasons (House 1997), but for many species it normally reaches a few dozen seeds. The reproductive output generally increases with tree age (Barbour et al. 2008). The development of mature fruits may take a few years (Cremer 1965b; Cunningham 1957; Grose 1960). Fruits normally remain on trees for several years and each individual tree may contain thousands of seeds (Ruthrof 2004). Eucalypt seeds are in general very small (1-3 mm) and light (less than 2 mg), and possess no obvious endosperm, meaning that newly emerged offspring depend strongly on cotyledon photosynthesis and a favourable substrate for their roots (Rejmánek & Richardson 2011).

Eucalypts do not form long-living soil seed banks. Instead, seeds are mainly kept in canopy seed banks (Cremer et al. 1984; Wellington & Noble 1985). Seed dehiscence occurs along the year, but there are peaks in summer, associated with hot periods (Cunningham 1957; Grose 1960). A massive, synchronised seed shed from eucalypts is triggered by wildfires (Cremer 1965a; Gill 1997; O'Dowd & Gill 1984). In Australia, practically all eucalypt species are subject to fire, even though with different frequencies and seasonality (Gill 1997). Fire is a vital disturbance to facilitate eucalypt recruitment. The post-fire environment favours the germination and growth of the young eucalypts by a number of reasons, including the elimination of competing vegetation and the satiation of seed predators (Vivian et al. 2008; Wellington & Noble 1985). Under normal conditions, seed predators, especially ants, may rapidly deplete dehisced seeds, thus severely limiting the chances of eucalypt recruitment (Andersen & Ashton 1985; Ashton 1979). Eucalypt seeds have no particular adaptations for distance dispersal, so that wind and water are the main dispersal agents (Cremer 1977).

6.3 Literature survey and overview

A literature survey, accomplished in September 2019, was aimed at gathering all studies investigating the natural establishment of eucalypts in the introduced range under field conditions (e.g. field surveys or experiments). It was targeted at any eucalypt species from the genera *Eucalyptus*, *Corymbia* and *Angophora*. The literature survey was conducted using the Web of Science Core Collection, an online database of scientific literature from peer reviewed journals, books and proceedings (Clarivate Analytics 2019). In the search engine of this platform, we used two sets of keywords targeted at ‘all fields’ (e.g. title; abstract) from publications: ‘eucalypt OR *Eucalyptus* OR *Corymbia* OR *Angophora*” (first field) and “invasion OR invasiveness OR recruitment OR natural establishment OR weed OR alien” (second field). The time span was “all years” (1990 – 2019). These search settings retrieved 1,262 references on the 24th of September 2019. Additionally, we checked personal bibliographic databases related to the topic to search for any missing publication.

Some publications probably went unnoticed, especially grey literature. One Master thesis (Díaz 2016) was included due to its relevance.

Our literature survey retrieved a total of 37 studies (32 from Web of Science), conducted in seven countries (Fig. 1), most of which published in the last years (Fig. 2). We should note that 'invasion ecology' is a relatively recent scientific field, which foundations were first established in the mid-20th century with the publication of 'The ecology of invasions by animals and plants' by C. Elton (Elton 1958; Richardson 2011a). Perhaps the first observation regarding eucalypt invasion dates from two years before this publication, in reference to the impacts of *E. globulus* in California (Oosting 1956, cited by Richardson 2011b). In 1975, in California and Arizona, J. B. Kirkpatrick probably performed the first systematic field surveys to investigate eucalypt recruitment in the introduced range (Kirkpatrick 1977). In those surveys, few, highly localised offspring from *E. camaldulensis*, *E. globulus* and *E. cladocalyx* were recorded. Between 1979 and 1993, a series of regional surveys were performed by L. Anderson in South Africa to record woody invasive plants along roads and watercourses, where nearly 20 eucalypt species were found to escape planted areas (Henderson 1989, 1991a, b, 1998; Henderson & Musil 1984). In the following years, up until very recently (year 2009), research on eucalypt invasion was scarce, with only four papers reporting field observations on eucalypt invasion (e.g. Forsyth et al. 2004; Ruthrof et al. 2003). Between 2011 and 2019, however, there was a multiplication of papers investigating the natural establishment of eucalypts, totalling 27 studies in seven countries (Fig. 1).

Most research was conducted in regions characterised by a Mediterranean-type climate (western cape of South Africa; western Australia; Central Chile; Portugal; California). Some research was also conducted in subtropical-type climates (Florida; SE Brazil), temperate-type climates (SW Australia), and throughout the contrasting biomes of South Africa (Fig. 1). Most studies were conducted in Portugal (n=9) and South Africa (n=8). These are also the countries most extensively surveyed, in part due to countrywide surveys (e.g. Catry et al. 2015; Henderson 1998). Extensive surveys were also performed in Australia (Larcombe et al. 2013) and California (Ritter & Yost 2009). In contrast, some studies were local and failed to record eucalypt offspring (e.g. da Silva et al. 2017; Lorentz & Minogue 2015).

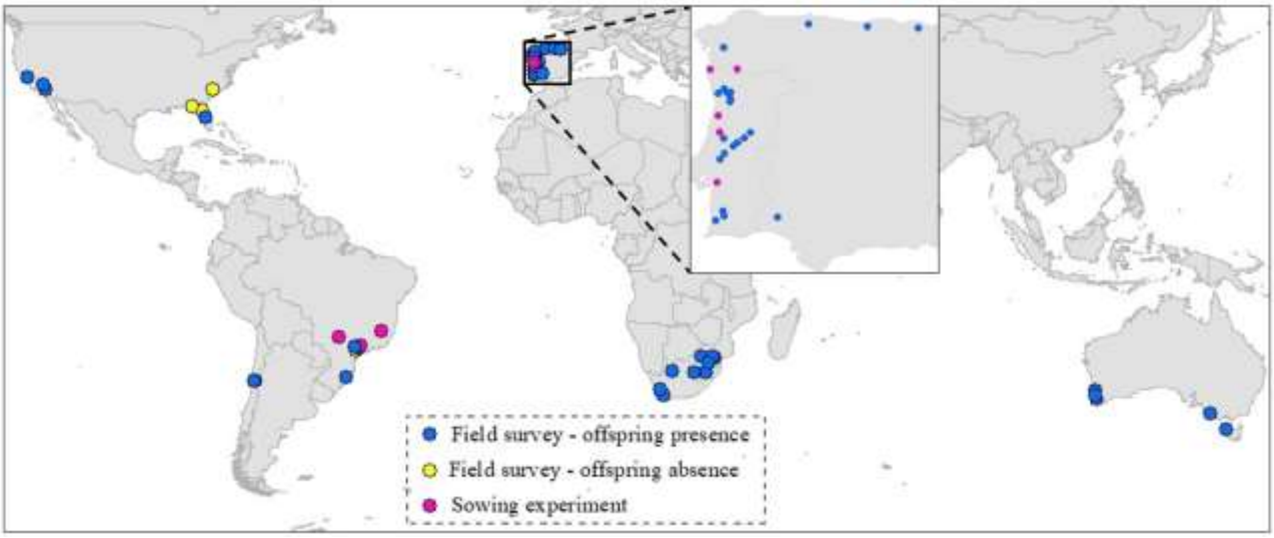

Fig. 1 - Location of studies (n=37) investigating the natural establishment of eucalypts in the introduced range through field observations. Studies are classified according to the type of study (‘field survey’ or ‘sowing experiment’) and, within the ‘field surveys’, the ‘presence’ or ‘absence’ of offspring. Studies using satellite imagery were included in ‘field surveys’. Studies correspond to more than one location if the study area includes different administrative regions (n=57 points). If the study performed both ‘field surveys’ and ‘sowing experiments’, we privileged ‘field surveys’. If the study surveyed several eucalypt species with different outcomes in the ‘presence’ or ‘absence’, we privileged ‘presence’.

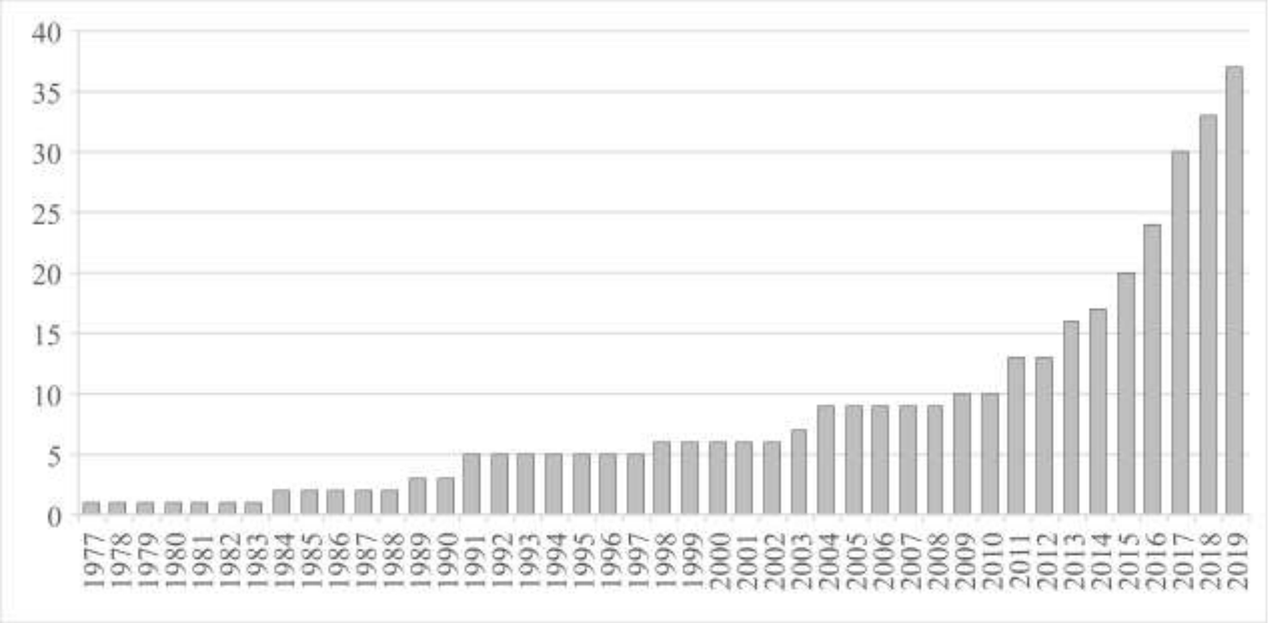

Fig. 2 - Cumulative number of studies performing field surveys or experiments to investigate the natural establishment of eucalypt species in the introduced range

The studies covered a total of 61 eucalypt species, including three hybrids (Table 1). We should stress, however, the large contribution by Ritter and Yost (2009) for surveying dozens of eucalypt species introduced to California. *Eucalyptus globulus* was the most investigated species, featured in 20 studies from six countries. Due to its importance, it was also the only eucalypt species investigated in Portugal, Spain and Chile. In contrast, observations in other countries, particularly Brazil, South Africa, USA (California and Florida) and Australia, focused on a wide array of eucalypt species. Other eucalypt species frequently found in the literature are *E. grandis* and *E. camaldulensis*, both figuring in 10 studies from three countries. The next most investigated eucalypt species were *E. cladocalyx*, *E. tereticornis*, *E. robusta* and *E. saligna*, which were the subject of 4-5 studies in 2-3 countries.

6.4 Offspring abundance and dispersal potential

6.4.1 General aspects

Offspring abundance and dispersal distances are two key factors liable to measurements that may provide some insights into the natural establishment and invasive potential of eucalypts. Despite the inexistence of a threshold on the number of naturally established plants, offspring abundance allows distinguishing casual (few plants) from naturalised species (many plants), while the distance of spread may help distinguishing naturalised from invasive species (Blackburn et al. 2011; Richardson et al. 2000b). Several studies provide data about offspring abundance and distance dispersal from eucalypts. Therefore, we tried to compute or retrieve these data from the set of studies surveyed to allow assessing and comparing the invasive potential of eucalypt species in different regions.

Studies used different protocols, which required an attempt to harmonise the results in order to provide meaningful comparisons among studies. For instance, some studies performed ‘random surveys’ while others surveyed ‘presence-only’ areas. In this case, we retrieved or calculated values of wildling densities only for ‘presence areas’, thus discarding all observations or survey areas where eucalypt offspring, for any reason, did not find conditions to establish.

Table 1 - List of eucalypt species subject of studies investigating the natural establishment in the introduced range through observations in field conditions (alphabetic order)

Eucalypt species	Eucalypt species	Eucalypt species
C. citriodora	*E. grandis*	*E. pulchella*
C. torelliana	*E. gunnii*	*E. pulverulenta*
E. amplifolia	*E. kitsoniana*	*E. punctata*
E. amygdalina	*E. lehmannii*	*E. radiata*
E. badjensis	*E. leucoxylon*	*E. regnans*
E. benthamii	*E. macarthurii*	*E. resinifera*
E. blakelyi	*E. macranda*	*E. robusta*
E. botryoides	*E. maculata*	*E. rubida*
E. calophylla	*E. mannifera*	*E. rudis*
E. camaldulensis	*C. citriodora*	*E. saligna*
E. cladocalyx	*E. megacornuta*	*E. sideroxylon*
E. conferruminata	*E. melliodora*	*E. spathulata*
E. cornuta	*E. neglecta*	*E. tereticornis*
E. dalrympleana	*E. nicholii*	*E. torquata*
E. diversicolor	*E. ovata*	*E. urophylla*
E. dives	*E. paniculata*	*E. viminalis*
E. dorrigoensis	*E. parvula*	*E. viminalis*
E. erythrocorys	*E. pauciflora*	*E. megacornuta*
E. fastigata	*E. pellita*	*E. globulus* x E. spp
E. ficifolia	*E. polyanthemos*	*E. urophylla* x *E. grandis* (*E. urograndis*)
E. globulus	*E. preissiana*	*E. dunnii* x E. spp

Other factor of discord between studies was the location of the surveys relatively to plantation limits. The survey location is of uppermost importance by determining the amount of wildlings one is expected to find. First, eucalypt species have a small seed shadow, meaning that propagule pressure is expected to drop sharply a few meters away from mother-trees (Calviño-Cancela & Rubido-Bará 2013; Cremer 1977). Secondly, there are contrasting biotic and abiotic conditions between locations, for instance between the interior of plantations, the plantation edges and adjacent habitats (Gehlhausen et al. 2000; Young & Mitchell 1994). Therefore, we retrieved or computed wilding densities according to five 'recruitment zones': a) *inside plantation*; b) *inner edge*; c) *outer edge*; d) *outside plantation 15-50 m*; e) *outside plantation 50-100 m* (Fig. 3).

Forest edges are of particular importance and should separate the inside from the outside of plantations. First, edges represent the point of potential escape from plantations (Deus et al. 2019). Second, flowering (and reproductive output) in eucalypt plantations is probably higher at the edges (Barbour et al. 2008). Third, edges form a transition zone featuring particular biotic (e.g. species diversity) and abiotic (e.g. microclimate) conditions (Murcia 1995). Fourth, most seed shed will likely occur in the edge of eucalypt plantations (Calviño-Cancela & Rubido-Bará 2013). Normally, there is no clearly delimited boundary for forest edges. The 'edge' width varies along time and space, as it depends on factors such as the type of adjacent ecosystems, plantations characteristics (e.g. tree height) or topography (Hatfield et al. 2019; Young & Mitchell 1994). The width of 15 m to delimit the edges of plantations was chosen because many studies surveying eucalypt wildlings delimit a zone of 10-15 m on either side of the plantation limits (e.g. Callaham Jr et al. 2013; Deus et al. 2019).

Studies investigating dispersal distances from eucalypt plantations normally perform surveys up to a distance of 80-100 m from the plantation limits. Since studies use different distance classes and survey lengths, we defined two 'recruitment zones' outside plantations to make a distinction between short-distance (*15-50 m*) and long-distance spread (*50-100 m*). When applicable, for each study, we averaged the observations within each 'recruitment zone'. Additionally, for the studies estimating offspring abundance across a gradient of distance from plantations, we retrieved data of wildling densities for each distance class, in order to generate recruitment curves. For the reasons previously explained, recruitment curves also considered exclusively 'presence-only' areas.

6.4.2 Offspring abundance

The surveyed studies show a wide amplitude of wildling densities in the 'recruitment zones' *inside plantation*, *inner edge* and *outer edge*. In other words, studies show contrasting results in offspring abundance near mother-plants, suggesting the interplay of different factors, such as: different studied eucalypt species, plantation ages (or propagule pressure), land covers or silvicultural practices. In contrast, *outside plantations* (*15-50 m*; *50-100 m*), the dispersion (amplitude) of the recorded densities is much smaller, since wildling densities are generally very low (Fig. 4). This consensus between studies suggests that a reduced dispersal potential is the main factor determining a reduced offspring abundance farther from plantations. In fact, taking into account all studies, there is a general trend of decreasing wildling densities from the *inside plantations* (mean=1,812.4 wildlings ha^{-1}; SE= 1,016.6) to the areas distanced *50-100 m* from plantation limits (mean=5.8; SD= 3.0).

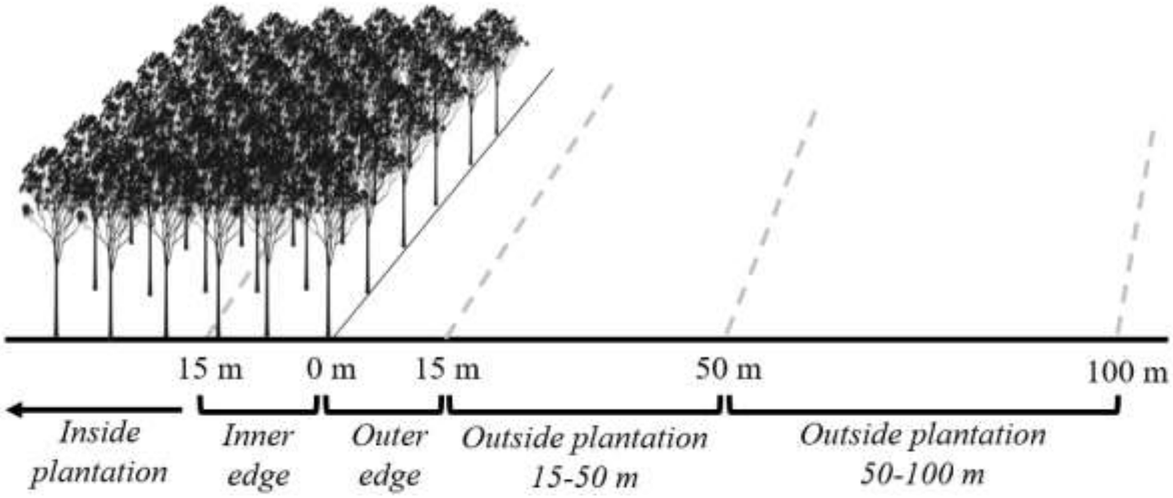

Fig. 3 - The five 'recruitment zones' relatively to the plantation limit (0 m) for which data on eucalypt offspring abundance was collected

The highest wildling densities were found '*inside plantations*' in recently burnt areas. That is the case of studies conducted in burnt stands of *E. megacornuta* and *E. cladocalyx* in western Australia (Ruthrof 2004; Ruthrof et al. 2003), and burnt *E. globulus* plantations in Portugal (Águas et al. 2014), registering wildling densities between 2,610 and 5,200 wildlings ha^{-1}. We should note that the Australian studies were performed in an urban park in Perth (Australia). They were included in the zone 'inside plantation' because it was suggested that the surveys were conducted near mother-trees. In contrast, the lowest value was 240.0 wildlings ha^{-1}, recorded inside one (out of three) *E. saligna* plantation surveyed in southeast Brazil (Emer & Fonseca 2011).

For all five studies comparing the abundance of eucalypt offspring between the *inner* and *outer edges* (Águas et al. 2017; Callaham Jr et al. 2013; Deus et al. 2019; Fernandes et al. 2016; Larcombe et al. 2013), it is consensual that eucalypt wildlings are more abundant in the *inner edges* of plantations (Fig. 4). Perhaps biotic and abiotic conditions are more favourable to eucalypt recruitment in the *inner edges*, but the existence of management, disturbances, roads or firebreaks in the *outer edges* of plantations may also artificially limit natural establishment at these locations (e.g. Calviño-Cancela & van Etten 2018).

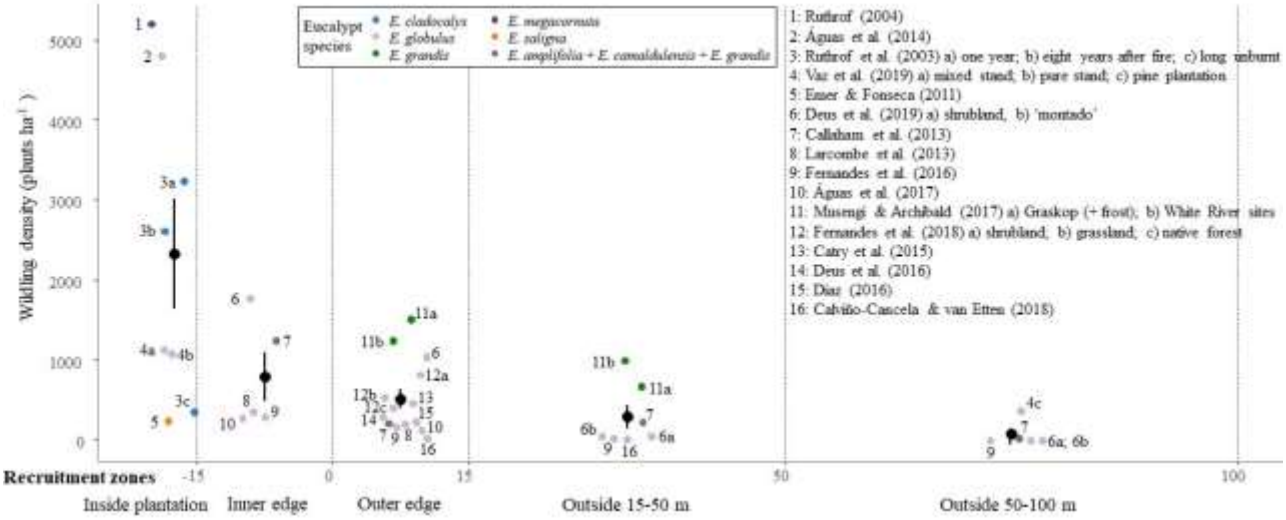

Fig. 4 - Wildling densities (wildlings ha^{-1}) retrieved from different studies for different 'recruitment zones' relatively to plantation limits and according to species (identified by colour). Please note that, in each study, data was retrieved for ‘presence-only’ areas, thus discarding areas where wildlings were not found. We averaged observations within a ‘recruitment zone’ when the study provided more than one estimate for that distance interval. Some values may not be accurate. Please see the papers for details. The references can be seen in the ‘References’ section. Points were moved horizontally in order to distinguish overlapping points. The average and standard error of wildling densities are presented for each ‘recruitment zone’

6.4.3 Dispersal potential

Recruitment curves produced from different studies show, in general, a sharp decrease of wildling densities along with distance from plantations (Fig. 5). This pattern is in accordance with observations from seed traps established outside *E. globulus* plantations in northwest Spain (Calviño-Cancela & Rubido-Bará 2013). This sharp drop of wildling densities reflects the lack of adaptation of eucalypt seeds for long-distance dispersal, which becomes clear when comparing dispersal between eucalypt and wind-dispersed pine seeds (Calviño-Cancela & van Etten 2018; Fernandes et al. 2016).

In Fig. 5, for most studies, the decline of wildling densities is not constant along with distance, since there is an ‘artificial drop’ in wildling densities in the first distance class outside plantations, at 0-10 m (Calviño-Cancela & van Etten 2018; Deus et al. 2019; Musengi & Archibald 2017) or 0-15 m (Callaham Jr et al. 2013). This is probably caused by the existence of roads, tracks or firebreaks that limit plant establishment. Firebreaks adjacent to plantations are very common in countries such as Australia and South Africa (Larcombe et al. 2013; Musengi & Archibald 2017). Nevertheless, natural establishment occurred, in some cases abundantly, beyond such narrow ‘safety belts’, suggesting that roads and cleared margins, alone, are not sufficient to contain the escape from plantations.

In Portugal, Fernandes et al. (2016) and (Deus et al. 2019) established survey transects perpendicular to *E. globulus* plantations up to 105 and 80 m from plantation limits, respectively. Both studies recorded wildlings spread up to a distance of 75-76 m (Fig. 5), despite lower densities outside plantations estimated by Fernandes et al. (2016), which should be partly explained by the fact that nearly half of the surveyed plantations were established in a region (interior Central Portugal) where *E. globulus* recruitment is lower (Catry et al. 2015). Another possible explanation is the fact that Fernandes et al. (2016) restricted the survey to areas of reduced slope (0-10%), because steeper slopes may enhance *E. globulus* recruitment by reducing competing vegetation and waterlogging (Catry et al. 2015). Also, from a geometrical perspective, seeds dispersed from trees located at higher ground can reach greater distances.

In contrast to the observations in Portugal, in western Australia, surveys outside *E. globulus* plantations by Calviño-Cancela and van Etten (2018) did not find wildlings distanced more than 20 m from plantations (Fig. 5). We should note, however, that the surveyed plantations were planned for very short rotations (8-10 years), before trees reaching their full reproductive potential, which may explain the limited recruitment. In fact, researchers found very few capsules in the canopy and on the ground. In another study investigating the natural establishment of *E. globulus* in Australia, wildlings were seldom found at distances greater than 10 m from plantations, but in one location the maximum distance recorded was 175 m (Larcombe et al. 2013). This study was not included in Fig. 5 due to insufficient data.

In Florida (USA), Callaham Jr et al. (2013) established survey transects up to a distance of 75 m from plantations of different eucalypts species. They recorded wildlings of *E. amplifolia*, *E. grandis* and *E. robusta* spread up to 75 m from plantations. Despite relatively high wilding densities inside plantation, wildling abundance also dropped sharply outside plantations (Fig. 5).

In the province of Mpumalanga, South Africa, Musengi and Archibald (2017) performed surveys outside *E. grandis* plantations. However, survey transects extended only up to 50 m from plantations. Nevertheless, the recruitment curves produced for two locations (White River and Graskop), registered wildling densities of c. 260 and 600 wildlings ha^{-1} at a distance of 40-50 m from plantations (Fig. 5), suggesting that eucalypt wildlings easily surpassed the limit of the survey transect.

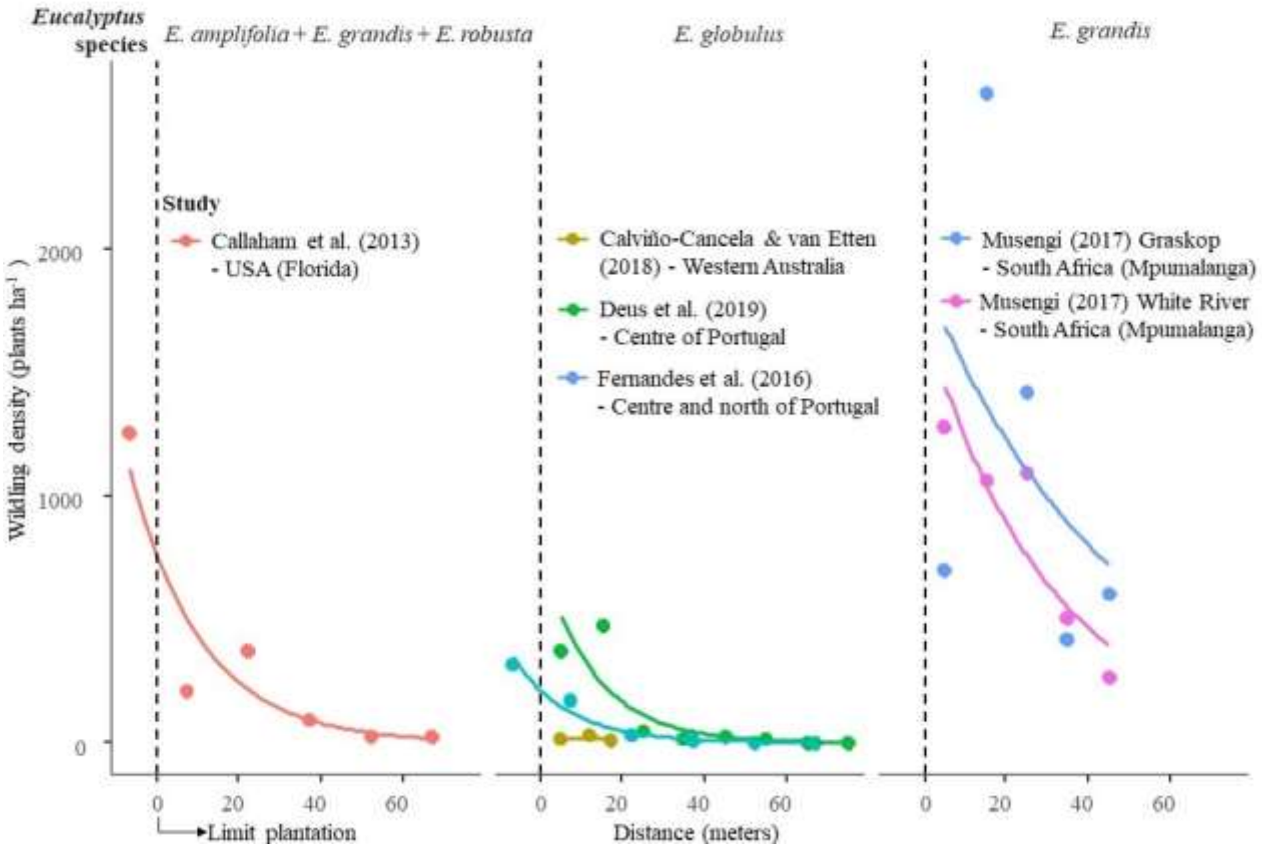

Fig. 5 - Dispersal curves produced from studies estimating the escape of different eucalypt species from plantations. The values of wildling density (wildlings ha^{-1}) were calculated for ‘presence-only’ areas, i.e. locations (plots or transects) with no wildlings were discarded. Data from Callaham et al. (2013) results from pooled observations from different eucalypt species

6.5 A review of natural establishment in different regions

6.5.1 General aspects

Research on the natural establishment of eucalypts and their invasive potential was conducted in different regions of the world. These regions contrast between each other in many ways, featuring contrasting climatic conditions, different biomes, different eucalypt species cultivated (and investigated) and different management practices. Therefore, in this section, we review the researches made and the most relevant findings in the different regions where studies were conducted. In section 6.6, we try to synthesise some of the most relevant factors and processes influencing eucalypt recruitment and their invasive potential.

6.5.2 The particular case of *Eucalyptus globulus*

Eucalyptus globulus, naturally occurring in Victoria (Australia), western Tasmania and the Bass Strait Islands (Kirkpatrick 1975), is one of the most widely cultivated eucalypt species (Potts et al. 2004). Also, it is the eucalypt species flagged as invasive in the greater number of world regions (Rejmánek & Richardson 2013), and the species which has been subject to most intensive and detailed research regarding invasiveness. Our literature survey identified 21 studies in seven countries addressing *E. globulus*, even though the most intensive research was conducted in Portugal.

6.5.2.1 Portugal vs. Australia

Research on *E. globulus* invasiveness has been extensively conducted across Portugal and Australia, with a replication of survey protocols which allows a fair comparison between these two disparate regions. Survey protocols first applied by Larcombe et al. (2013), on the edges of *E. globulus* plantations across Australia and Tasmania, were later replicated in Portugal by Águas et al. (2017) and Deus et al. (2019).

Águas et al. (2017) estimated that the overall wildling density was c. 15 times higher in Portugal than in Australia (comparatively to Larcombe et al. 2013), even though this ratio reduced to c. 3 times when restricting the survey to unburnt, first rotation plantations. Deus et al. (2019) replicated a ‘small-scale survey’ from Larcombe et al. (2013), by establishing plots in locations featuring a minimum of five wildlings, and confirmed that wildling densities were several times higher in Portugal (Fig. 4).

Results from both countries suggest also differences in wildling phenology and demography. In Australia, Larcombe et al. (2013) found that more than half of the c. 5,000 wildlings surveyed were taller than 3 m, even though only a few had adult foliage and none was reproductive, i.e. did not possess flowers or fruits. In contrast, in Portugal, Deus et al. (2019) recorded a balanced distribution of wildlings' size classes, suggesting a continuous recruitment along time. Besides, they found that nearly 8% of the surveyed wildlings (out of 1,630 surveyed wildlings) were reproductive. Statistical analysis revealed that reproductive wildlings were more abundant in the *outer edges* of plantations, probably due to lower competition for light and nutrients (Williams et al. 2006). Likewise, survey transects outside plantations performed by Deus et al. (2019) showed that more than half (59%) of the surveyed wildlings possessed adult foliage, meaning that they overcame the most critical stages of survival and can become reproductive. Moreover, they recorded some very small reproductive wildlings (less than 1.5 m tall). Findings in Portugal suggest that wildlings escaped from plantations not only overcome the most critical stages of survival, but are also more likely to become reproductive, and that the species may have a very short juvenile period, a trait that is often associated with invasive species (Grotkopp et al. 2002; Rejmánek & Richardson 1996).

6.5.2.2 Research in southwest Europe

In Europe, to our knowledge, research on eucalypt invasiveness was restricted to the Iberian Peninsula, southwest Europe, particularly in Portugal and Spain. All research focused on *E. globulus*, despite records of *E. camaldulensis* invasion in Spain (Dana et al. 2004). Countrywide surveys in Portugal and Spain revealed the extraordinary capacity of *E. globulus* to originate offspring throughout most of the introduced range, across a wide range of environmental conditions, despite some regional variations (Catry et al. 2015; Deus et al. 2016; Díaz 2016). It seems that, in Portugal, a particularly favourable combination of factors enhance the natural establishment of *E. globulus*, in particular the massive expansion and cover of *E. globulus* in some regions (Alegria et al. 2019; Deus et al. 2018a), the abandonment of *E. globulus* plantations (Silva et al. 2011; Vaz et al. 2019), the recurrent wildfires (Nunes et al. 2016) and land abandonment, including agricultural areas (Silva et al.

2011). The same factors apply at a lower degree to some regions of Spain where the species is regarded as invasive, even though research has been much less intense.

In Central and North Portugal, Águas et al. (2014) recorded exceptional wildling densities in burnt *E. globulus* plantations, 5-7 years after fire (4,800 wildlings ha^{-1}; Fig. 4). Likewise, in northwest Portugal, Vaz et al. (2019) estimated 1,075.0 wildlings ha^{-1} inside stands dominated by *E. globulus* and 1,134.6 wildlings ha^{-1} inside mixed stands featuring the native *Pinus pinaster* (Fig. 4). This study provides an estimate of 363 wildlings ha^{-1} inside stands dominated by *P. pinaster*. It was not possible to know where offspring originated, but survey areas were established in the centroid of the surveyed pine stands, so wildlings may have escaped from nearby plantations or perhaps from isolated trees (in Fig. 4 this estimate was allocated to '*outside plantations 50-100 m*'). No fire history is provided, but it is quite likely that some plantations experienced fire given the fire regime in this region (Nunes et al. 2016), which may have facilitated the invasion. Águas et al. (2014) recorded an average wildling height of 2.7 m (SD=2.4), ranging from 0.2 to 12.1 m, including some reproductive wildlings. Vaz et al. (2019) measured diameters at breast height (DBH) greater than 30 cm, even though for the estimation of offspring abundance in Fig. 4 we excluded trees with DBH > 20 for a conservative estimate.

Surveys inside (Águas et al. 2014; Vaz et al. 2019) and outside (Deus et al. 2019; Fernandes et al. 2016) *E. globulus* plantations in Portugal provide solid evidence that the species is naturalised and it can successfully invade adjacent habitats (see section 6.4.3), even though other studies indicate that different habitats adjacent to plantations have different levels of invasibility. A sowing experiment in NW Spain showed that native forests are more resistant to the emergence and survival of *E. globulus* wildlings compared to shrubland and pine plantations (Calviño-Cancela & Rubido-Bará 2013). This was later confirmed in field surveys across Portugal. Fernandes et al. (2018) found higher offspring abundance in shrublands compared to native forests or grasslands.

In Fig. 4, however, the estimated wildling densities for native forests and grasslands (533 and 400 wildlings ha^{-1}) seem relatively high because they were retrieved from a small number of 'presence-only' areas (only five survey plots out of 624; total of 12 wildlings recorded). In contrast, the average density of c. 814 wildlings ha^{-1} for shrublands was retrieved from 115 survey plots (12% of plots; 468 wildlings recorded). Deus et al. (2019), however, found similar wildling densities in shrublands and 'montados' (a traditional agroforestry system dominated by native oaks), reaching c. 55.6 and 44.8 wildlings ha^{-1} at *15-50 m*, and 5.0 and 2.6 wildlings ha^{-1} at *50-100 m*, respectively (Fig. 4).

6.5.2.3 Reports from California

In the USA, research on the natural establishment of *E. globulus* was restricted to California, where the species is widespread and has a long history (Doughty 2000). Today, *E. globulus* is no longer planted nor seeds sold in California (Wolf & DiTomaso 2016). The first surveys by Kirkpatrick (1977) in California showed that natural establishment was mostly limited to bare ground sites and wildlings were seldom found at more than 20 m from parents. There are, however, many reports that *E. globulus* can escape from plantations with concerning impacts (Wolf & DiTomaso 2016). Extensive surveys across California led Ritter and Yost (2009) to classify *E. globulus* as ‘naturalised’ according to the conceptualisation by Richardson et al. (2000b). We found no quantitative data of wildling abundance but some studies provide rare insights into the expansion of eucalypt stands along time. In Central California, Fork et al. (2015) analysed aerial imagery between 1931-2001 for six *E. globulus* stands and estimated a mean rate of expansion of 271% during that period. Similarly, in Angel Island (San Francisco Bay, California), between 1915 and 1988, *E. globulus* stands expanded 3.7 times, from 0.24 to 0.89 ha, up to a distance of 98 m from the original locations (McBride et al. 1988).

6.5.2.4 Unlikely invasion in Central Chile

Central Chile is characterised by a semiarid climate, with an average of 330 mm precipitation concentrated in 5-6 months, opposed to a 6 or 7-month drought period. This is a much harsher environment than the harshest where *E. globulus* is found in the native range, registering 500 mm of precipitation evenly distributed along the year, confining *E. globulus* to southern aspects and along streams (Kirkpatrick 1975). In Central Chile, near Santiago, Becerra (2006) surveyed alien invasive plants in a small watershed (c. 2 x 1.5 km), recording and measuring all individuals that were planted or naturally established. A total of 50 *E. globulus* wildlings were recorded (22 planted), most of which taller than 2 m, with diameters at breast height up to 30 cm, at distances greater than 100 m from parent plants. The dry environment explains why all *E. globulus* wildlings were located in the south-facing slope (lower solar radiation in southern hemisphere) and the valley bottom. Several *E. camaldulensis* wildlings were also found, exclusively in the valley bottom. This is probably the driest region where *E. globulus* was found to thrive and it shows how local factors, particularly aspect, can overshadow unfavourable climate. A sowing experiment performed later in the same region showed the importance of aspect and shadow for *E. globulus* germination and survival in this stressful environment (Becerra & Bustamante 2011).

6.5.3 Low recruitment in Brazil

Weed risk assessments for 16 eucalypt species cultivated in Brazil, selected seven species with high risk, eight species with moderate risk and only one species with low risk (Ziller et al. 2019). Studies investigating the natural establishment of eucalypts in Brazil were restricted to a large region in the southeast (Fig. 1), where many eucalypt plantations have been established. Brazil has experienced the largest expansion of eucalypt plantations in the last decades. Between 2006 and 2017, the cultivated area practically doubled to 7.4 million ha (SNIF 2019). Several eucalypt species and their hybrids are widely planted in Brazil, including *E. grandis*, *E. urophylla*, *E. saligna*, *E. camaldulensis*, C. citriodora and *E. urophylla* x *E. grandis* (*E. urograndis*) (Gonçalves et al. 2013). There is a general agreement on the poor invasive potential of eucalypts in Brazil. Many eucalypt species were found to originate offspring, such as *E, grandis*, *E. urophylla, C. citriodora*, *E. grandis* and *E. saligna*, but normally recruitment is scarce, localised and confined to plantations or nearby areas (Emer & Fonseca 2011; Miolaro et al. 2017).

The reduced recruitment in Brazil should be explained by many factors, including a reduced habitat invasibility due to a higher ‘ecological resistance’ (Elton 1958) in competitive (sub)tropical environments, among other biotic and abiotic factors (Rejmánek 1996). A lower propagule pressure may be important since eucalypt plantations can have the shortest rotations in Brazil (6-8 years; Gonçalves et al. 2013). Clonal plantations are particularly popular in Brazil. A low genetic variability may reduce the probabilities of naturalisation (Lockwood et al. 2005) by means of self-pollination, resulting in limited seed production and seedling survival (Potts & Gore 1995). In Brazil, da Silva et al. (2017) investigated the natural establishment (and pollen flow) of hybrid clones of *E. urograndis* (*E. urophylla* x *E. grandis*) in one experimental plantation. These cloned hybrids reveal an exceptionally short juvenile period, by producing seeds 18 months after planting. However, no wildlings were found inside or outside (up to 100 m) the plantation surveyed, along a monitoring period of four years. It is worth noting that, following biosafety regulations, the surveyed plantation was surrounded by a 100 m wide grassland safety belt composed of *Brachiara* sp.

In SE Brazil, Miolaro et al. (2017) surveyed several seed orchards of different eucalypt species, up to 30 m from plantations. Some offspring was found in a few plantations (max. 29 wildlings), mostly concentrated at plantation edges. Nevertheless, most wildlings were taller than 1 m, suggesting that eucalypt wildlings can survive outside plantations. Recruitment was localised in bare ground areas, burnt sites and areas gathering wastes from seed harvesting. In this case, trees have been producing seeds for more than 12 years and tree height ranged from 30 to 60 m, meaning that seed output is probably much greater than in typical short-rotation plantations.

In a conservation area of Araucaria forest in SE brazil, no wildlings were found outside *E. saligna* plantations (Emer & Fonseca 2011). Instead, eucalypt wildlings were found in relatively low densities (240 wildlings $ha^{-}1$) inside one of the three plantations surveyed (Fig. 4). The reduced recruitment may be partly explained by the ecologically-oriented management of monocultures in this reserve (Fonseca et al. 2009). Sowing experiments using different eucalypt seeds confirm low germination rates, with a preference for (artificially) disturbed areas, followed by high mortality rates (da Silva et al. 2016; da Silva et al. 2011).

6.5.4 Invasive eucalypts across South Africa

South Africa is probably one of the countries where most extensive surveys for alien invasive plants was done. Research on eucalypt invasion in South Africa was mostly performed within the scope of projects to detect and prioritise invaded areas for control actions. Most studies consisted on field surveys along roads, railways, river stretches and in water crossings (e.g. Forsyth et al. 2004; Henderson 1998; Mararakanye et al. 2017). Data on eucalypt recruitment consists mostly of records of presence and frequency, and abundance was mostly assessed using qualitative scales (e.g. Henderson 1991a; Henderson 1991b).

A set of studies performed surveys along roadsides and watercourse crossings across South African regions (Henderson 1989, 1991a, b, 1998; Henderson & Musil 1984). Several invasive eucalypt species were detected, some of which reported as invasive in other countries, such as *E. camaldulensis*, *E. globulus*, *E. grandis*, *E. cladocalyx*, *E. tereticornis* and *E. robusta*. Watercourses provide a reliable mean of spread for different eucalypt species, especially *E. camaldulensis*, which invaded heavily some streambanks (Henderson 1991a, 1998). Watercourses may allow some eucalypt species to thrive in the stressful conditions of arid environments (Henderson 1991a). In Mpumalanga (eastern South Africa), analyses of satellite images showed that railways promote the dispersal of eucalypts, which are spreading to and from adjacent agricultural lands (Mararakanye et al. 2017).

Forsyth et al. (2004) surveyed rivers stretches in two South African provinces to assess eucalypt invasion based on the five-stage model conceived by Richardson et al. (2000b). They found that several riverine habitats were invaded by *E. grandis* and, especially *E. camaldulensis*, which was classified as a ‘transformer’ due to the striking transformation of the invaded habitats. Other eucalypt species were considered as ‘naturalised’, namely *E. sideroxylon*, *E. paniculata* and *E. lehmannii*.

Only one study provided data on offspring abundance. In Mpumalanga, Musengi and Archibald (2017) surveyed natural velds adjacent to *E. grandis* plantations. Surveys were performed at two locations distinguished by frost frequency: Graskop (more frost) and White River (less frost). These surveys provided the highest wildling densities recorded outside plantations, reaching c. 1507 and 1241 wildlings ha^{-1} within *outer edges* (0-15 m), and c. 1,000 and 668 wildlings ha^{-1} within 15-50 m from plantations, in Graskop and White River, respectively (Fig. 4). We should note, however, that survey transects were established purposely in locations enclosing a large number of wildlings in order to assess wildling demography and phenology. They found a wide range of wildling sizes (max. 30 m tall), suggesting a continuous recruitment across time or the occurrence of several successful recruitment events. Also, they found large proportions of flowering wildlings, including some very small wildings, meaning that the species can have a short juvenile period.

6.5.5 Other eucalypt species in the USA

The application of the Australian weed risk assessment on eucalypt species introduced to the USA revealed that 14 eucalypt species have high probability of invasion (Gordon et al. 2012), including eucalypt species regarded as invasive elsewhere, such as *E. globulus*, *E. camaldulensis*, *E. grandis* and *E. cladocalyx*. In the USA, research on eucalypt invasiveness consisted of field surveys conducted in the west coast (California) and the southeast coast (South Carolina and Florida).

Ritter and Yost (2009) made field observations in stands and small plantings across most of California to classify invasiveness following the criteria by Richardson et al. (2000b). A total of 18 species were classified as ‘naturalised’, including nine of the 14 species ranked previously by Gordon et al. (2012) as having high probability of invasion. Eight other species were not assessed in the weed risk assessment by Gordon et al. (2012) while another needed further evaluation.

In Florida (USA), Callaham Jr et al. (2013) surveyed plantations from different eucalypt species but recruitment occurred exclusively in plantations from *E. amplifolia*, *E. grandis* and *E. robusta*. The *inner edges* showed relatively high wildling abundance, reaching 1,250.0 wildlings ha^{-1} (Fig. 4 and Fig. 5), even though these estimates took into account ‘presence-only’ areas, which included of 48 ‘presence’ transects (10% of survey transects). We should note that, in this study, most wildlings originated from seed orchards, where trees are managed to maximise seed production. Also, outside plantations, wildlings were concentrated in unmanaged areas like ‘failed eucalypt plantations’, while no wildlings were found in intensively managed areas like agricultural areas, suburban areas and citrus orchards. In contrast, Lorentz and Minogue (2015) also performed surveys inside and outside (up to 60 m) an *E. amplifolia* seed orchard in central Florida and a *E. amplifolia* stand in north Florida, but they did not find any offspring despite stands had been producing seeds for several years.

6.5.6 Eucalypts behaving invasively in Australia

Eucalypts may also reveal an invasive behaviour in Australia when introduced to areas outside the native regions, either as ornamentals or as planted forests. Virtue and Melland (2003) performed a weed risk assessment for eucalypt species normally used in forestry in South Australia. Most species posed negligible risk, namely *E. globulus*, *E. grandis*, *E. occidentalis*, *E. platypus* and *E. saligna*, even though there are anecdotal reports of occasional escape from plantations elsewhere in Australia (Virtue & Melland 2003). Only *E. cladocalyx* was considered to have high risk of invasion, due to the ability to establish more readily in native vegetation.

There are reports of invasion by *Corymbia torelliana* in the Australian east coast, where the species has been widely planted (Wallace & Trueman 1995). This species has the particularity of being the only eucalypt species dispersed by stingless bees, by foraging for resin inside open capsules. There are reports of seeds deposited on bee hives up to distances of 300 m from the closest tree (Wallace et al. 2007; Wallace & Trueman 1995).

Eucalypt species introduced as ornamentals can also be successful invaders in Australia. This is the case of *E. megacornuta* and *E. cladocalyx* which were introduced as ornamentals in the 1930's into the inner city Kings Park, in Perth (western Australia). Field surveys showed that both *E. megacornuta* and *E. cladocalyx* can produce abundant offspring, especially in recently burnt areas (Ruthrof 2004; Ruthrof et al. 2003). Densities were estimated at around 5,200 (*E. megacornuta*, twelve years after fire) and 3,220 (*E. cladocalyx*; one year after fire) wildlings ha^{-1} (Fig. 4). Eucalypt wildlings from both species covered all size classes, including individuals taller than 10 m, but size structure varied according to time since fire, with much more juveniles in recently burnt areas. The authors hypothesise that canopy seed store of these introduced species is higher than in native eucalypts, especially in the case of *E. cladocalyx*, explaining why it originates higher densities than *E. megacornuta* (Ruthrof 2004). It is estimated that *E. cladocalyx* spread up to 70 m from its original locations (Ruthrof et al. 2003). It seems that offspring are subject to high levels of mortality since offspring abundance reduces substantially along with time since fire (Ruthrof et al. 2003). According to Ruthrof (2004), in Kings Park, C. J. Yates recorded higher wildling densities for other eucalypt species after one wildfire in 1989: 5,760, 9,440 and 14,400 wildlings ha^{-1} for *E. botryoides*, *C. citriodora* and *C. cladocalyx*, respectively (unpublished data).

6.6 Factors influencing recruitment

6.6.1 Regional factors

Most reports of eucalypt invasion concern recruitment from plantations, i.e. locations where trees are intentionally established for exploitation. Eucalypt species cultivated in a certain region are normally selected for being physiologically well adapted to local conditions, particularly climate, in order to assure the best performance and outcomes (Richardson 1998). Silvicultural management, tree breeding programs and the use of hybrids can also enhance performance substantially, and allow eucalypts to thrive in novel climatic niches (Gonçalves et al. 2013; Wingfield et al. 2013). Exotic trees selected for cultivation possess traits such as environmental adaptation, fast growth and resistance to pests, which may enhance their invasive potential (Dodet & Collet 2012). It is thus expected that planted eucalypts find good conditions to thrive in the introduced ranges, but not necessarily to become invasive.

It is worth noting that plantations benefit from a manipulation of factors not shared by wild eucalypts. For instance, plantations are normally established with large, nursery-developed seedlings, planted at the most favourable season, on seedbeds prepared free from competition, sometimes irrigated and/or fertilised (Gonçalves et al. 2008). In contrast, naturally established eucalypts must have the capacity to germinate and survive along the most vulnerable stages. In Brazil, for instance, eucalypt plantations reach very high productivity (Gonçalves et al. 2013; Gonçalves et al. 2008) but natural establishment is severely conditioned (da Silva et al. 2016; da Silva et al. 2011). In Portugal, however, studies found a positive correlation between plantation productivity (based on climatic and soil parameters) and the invasiveness of *E. globulus* (Águas et al. 2014; Fernandes et al. 2018).

The similarity with native climate conditions has been widely used as a predictor of invasion (e.g. Panetta & Mitchell 1991; Thuiller et al. 2005). The native ecological range of eucalypt species is normally confined to a relatively narrow amplitude of mean temperature and precipitation (Hughes et al. 1996a). The natural distribution may not correspond to the whole ‘potential’ range, since species range is restricted by biotic factors (e.g. competition; predation), abiotic factors (e.g. physical barriers), historical events (e.g. glaciation) and species’ specific traits (e.g. limited dispersal). It is known that eucalypt species, including many regarded as invasive, can thrive under a broader array of climatic conditions (Booth et al. 1988), but species may respond in different ways to variation in environmental conditions, like in their reproductive behaviour or growth rate (Austin et al. 1983). For instance, in South Africa, *E. nitens* may not flower or produce seeds because it is grown under conditions warmer than its natural distribution (Booth 2012, pers. comm. by T. Swain).

Plant species can naturalise or invade in novel biomes not occupied in the native range (Gallagher et al. 2010; Richardson & Thuiller 2007). Some studies suggest that eucalypts with wider environmental tolerance are more likely to be planted outside the native range (*introduction*) and to reproduce successfully (*naturalisation* and *invasion*). Hui et al. (2014) showed that eucalypt species with larger native ranges are more likely to become naturalised (not invasive) in the introduced ranges. Gallien et al. (2019) concluded that eucalypts with a larger potential niche (worldwide) are more likely to become invasive. It is acknowledged, however, that the size of the native range does not always correlate to broader environmental tolerance. For instance, *E. camaldulensis*, whose native range covers more than half of Australia's mainland, is mostly confined to watercourses (Hughes et al. 1996b), similarly to invaded areas in South Africa (Forsyth et al. 2004; Henderson 1991a, 1998) and Chile (Becerra 2006).

In Portugal and Australia, *E. globulus* plantations are widespread across a range of environmental conditions, providing a large experimental area to explore the potential range and regional variation on natural establishment. In both countries, surveys across the introduced range allowed to depict significant regional differences on natural establishment potential, mostly determined by precipitation- and temperature-related variables (Catry et al. 2015; Deus et al. 2016; Larcombe et al. 2013). However, regional-scale variables, particularly climate, may have a too broader resolution to accurately locate invasions or invasion-prone areas. An illustrative example is the invasion of *E. globulus* in the semi-arid climate of Central Chile, where the species can spread across south-facing aspects and bottom valleys (described in section 6.5.2.4). Studies performing a multi-scale analyses, from regional to local factors, of eucalypt recruitment, show the importance, and sometimes preponderance, of site variables (Fernandes et al. 2018; Larcombe et al. 2013). Climate seems to operate especially at the first stages of the introduction-continuum invasion, by determining species introduction and conditions for germination and survival of new-born eucalypts. Local-scale factors, including species and plantation related traits, seem thus prevalent at later stages of the invasion continuum (Richardson & Pyšek 2012), in other words, 'populations, and not species, invade' (Richardson & Pyšek 2012, p. 384).

6.6.2 Local factors

Studies assessing the natural establishment of eucalypts at local-scales reveal that recruitment and invasion can be highly localised and dependent on a favourable combination of factors. Also, the potential for natural establishment is not constant along time. Propagule pressure seems to be a major issue (Lockwood et al. 2005). Plantations aimed at very short-rotations (6-8 years) seem to restrain eucalypts from their full reproductive potential (Calviño-Cancela & van Etten 2018). At the other end of the spectrum, mature seed orchards, aimed at maximising seed production, seem to be particularly prone to originate offspring (Callaham Jr et al. 2013; Miolaro et al. 2017). There seems to exist a correspondence with the rotation periods normally used in each country and the existence of reports of eucalypt invasion, where shorter rotations correspond to no reports (see the rotation period in Harwood 2014). Studies found a positive correlation between plantation age and the abundance of eucalypt offspring (Águas et al. 2017; Callaham Jr et al. 2013; Larcombe et al. 2013). Likewise, eucalypt recruitment may be enhanced near trees bearing greater number of capsules (Deus et al. 2019; Larcombe et al. 2013). In contrast, low levels of *E. globulus* recruitment observed by Calviño-Cancela and van Etten (2018) were associated with very few capsules in the canopy and on the ground. In general, it seems that as plantations grow older, reproductive output increases, as well as probabilities of recruitment.

Given the sensitive of new-born eucalypt wildlings to competition (Stoneman 1994), it is expected that land cover, particularly in what concerns competing vegetation, has a major effect on eucalypt recruitment. Studies suggest that eucalypt recruitment occurs preferentially on competition-free areas (Deus et al. 2019; Kirkpatrick 1977). Sowing experiments confirmed that rates of eucalypt germination and survival increased in disturbed areas as opposed to undisturbed, vegetated areas (da Silva et al. 2011). In Portugal, in areas adjacent to *E. globulus* plantations, Fernandes et al. (2018) found a positive relation between offspring abundance and the level of disturbance, which corresponded to human trampling, debris accumulation, logging and soil erosion. Competition may not always be detrimental. Under dry and hot periods, understorey may have a positive effect on ameliorating micro-climate conditions for new-born eucalypts by the effect of shadow (Becerra & Bustamante 2011).

Management operations on eucalypt plantations and surrounding may cause considerable disturbances on soil and vegetation, so it is likely that they may affect eucalypt recruitment. A recent study found that different types of standard management operations (harrowing; soil levelling; prescribed burning) can have different impacts on the germination and survival of new-born *E. globulus* wildlings (Nereu et al. in press). An interesting aspect is that all management operations had a negative effect on seeds previously shed on the ground, showing the vulnerability of seeds to burying and fire. However, for seeds shed after treatments, soil harrowing seemed to favour establishment, by destroying vegetation and by creating an irregular micro-topography that provided favourable micro-sites for the establishment of the new-born eucalypts. It is known that in the native range, microsite features such as depressions and shelters favour the retention of moisture and facilitate eucalypt establishment (Bailey et al. 2012; Battaglia & Reid 1993)

Despite a preference for competition-free areas, there are also reports of eucalypt establishment in different habitats such as shrublands, agro-forestry systems, wetlands, riparian areas and other eucalypt plantations (Callaham Jr et al. 2013; Deus et al. 2019; Fernandes et al. 2016; Forsyth et al. 2004; Musengi & Archibald 2017). However, some habitats, such as native forests and grasslands, revealed to be more resistant to eucalypt invasion (Calviño-Cancela & Rubido-Bará 2013; Calviño-Cancela & van Etten 2018; Fernandes et al. 2016).

Fire seems to be a major disturbance event able to disrupt the resistance of some habitats and aggravate the risk of invasion (Calviño-Cancela et al. 2018). It is probably not a coincidence that most studies on the invasive potential of eucalypts were performed within Mediterranean-type regions subject to recurrent wildfires. Eucalypts were introduced into many regions which experience recurrent wildfires, such as Portugal, Spain, Chile, California, South Africa and Australia. In some regions, there is a perception that the proliferation of eucalypt plantations aggravates fire regimes (Gómez-González et al. 2017; Mandle et al. 2011; McWethy et al. 2018; Moreira et al. 2009). In Portugal, for instance, wildfires may favour the abandonment of eucalypt plantations, allowing fuel to accumulate, which turns plantations more prone to fire, creating a feedback loop between fire occurrence and increased fire hazard (Silva et al. 2011). The exceptional eucalypt offspring abundance often seen in burnt areas (e.g. Águas et al. 2014; Ruthrof et al. 2003) is explained by a favourable post-fire environment for wildling establishment (Gill 1997), associated to a massive, fire-triggered dehiscence a few weeks after fire (Silva et al. 2016). Sowing experiments in burnt areas revealed that germination and survival of *E. globulus* is enhanced in burnt areas, and showed the importance of the destruction of litter that acts as a barrier to wildling emergence (Calviño-Cancela et al. 2018).

6.7 Further research

Some aspects that have been poorly investigated may help shed some light into the natural establishment of eucalypts in the introduced range. One key aspect frequently neglected is wildling development. Wildling development is of uppermost importance, particularly in what concerns the capacity of wildlings to survive and reproduce. It is known that eucalypt wildlings are subject to high levels of mortality in the first weeks (da Silva et al. 2011; Fernandes et al. 2017; Lorentz & Minogue 2015). In a sowing experiment using *E. globulus* seeds in field conditions, Calviño-Cancela and Rubido-Bará (2013) estimated that between the 6th and 8th months, survival probability was 82%. Therefore, wildling age or size may be a good indicator of wildling survival. The only reference we found regarding the growth of naturally established wildlings estimated an height of 1 m for six-month old wildlings (Burns & Honkala 1990). In the context of invasion, it is crucial to assess also the reproductive capacity of eucalypt wildlings escaped from plantations. Only a few studies recorded the existence of reproductive wildlings (Deus et al. 2019; Musengi & Archibald 2017), but no study tested wildling fertility and the existence of third- or above-generation wildlings.

The survival and mortality of eucalypt wildlings has been poorly investigated. Studies investigating demography normally use sowing experiments, where seeds are placed (or buried) and monitored for some months to assess germination, survival and the influence of particular factors such as shadow, management operations or disturbance (e.g. Becerra & Bustamante 2011; Fernandes et al. 2017; Lorentz & Minogue 2015; Nereu et al. in press). However, little is known about the long-term demography of naturally established eucalypts. It would be particularly interesting to understand the mortality, growth and the onset of sexual maturity.

Eucalypts are subject to intensive tree breeding programs to improve performance (Harwood 2014). Breeding programs are aimed at specific traits like growth and tolerance to pests and diseases, but the same traits, or some collateral effects on other traits, like improved seed production, may increase the risk of recruitment, but this issue remains poorly explored. Also, little is known about how genetic diversity of eucalypt populations can affect their invasive potential. The introduction and mixture of novel genetic material by the means of new cultivated areas can promote genetic variation and increase the chances of invasiveness (Lockwood et al. 2005). The recombination of genetic material can originate new phenotypical and physiological traits that enhance eucalypt invasiveness. Another possibility is interspecific hybridisation, allowing new individuals to inherit invasive traits (Schierenbeck & Ellstrand 2008). Eucalypt hybrids, resulting from manipulated hybridisation, are widespread in many countries as they revealed gains of productivity, but little is known how natural or manipulated hybridisation can enhance invasiveness. Research on the invasiveness of eucalypt hybrids was conducted in Brazil and results showed no invasiveness advantage relatively to pure races (da Silva et al. 2016; da Silva et al. 2011), even though hybrid clones of *E. urograndis* (*E. urophylla* x *E. grandis*) started producing seeds after 18 months (da Silva et al. 2017). Natural interspecific hybridisation within eucalypt genera is common and was soon registered after introduction of eucalypts outside the native range (Griffin et al. 1988). *Corymbia torelliana*, which behaves invasively in Australia, and is classified as a high risk invader in Brazil (Ziller et al. 2019), readily hybridises with other species, even though no reproductive advantages were found (Wallace & Leonhardt 2015). In Australia, *E. globulus* was also found to hybridise outside the native range with *E. camaldulensis* and *E. ovata*, and natural hybrid wildlings were found in one site (*E. globulus* x *E. ovata*) (Barbour et al. 2008). It is known that natural or manipulated hybridisation may allow new individuals to inherit invasive traits (Schierenbeck & Ellstrand 2008) so this is a topic worthy of future research.

A scarcity of natural enemies may help explaining the invasive behaviour of many alien species (Colautti et al. 2004; Keane & Crawley 2002). In Australia, predation of post-dispersed seeds is a major factor limiting eucalypt recruitment (Andersen 1982; Andersen & Ashton 1985; Wellington & Noble 1985), but the predation of eucalypt seeds in the introduced range has been poorly explored. A small experiment in Portugal revealed that *E. globulus* seeds are highly attractive to local fauna, including ants and rodents, but seed predation was highly localised, and seeds persisted in nearly half of the sites, which may help explaining the spatial heterogeneity in recruitment (Deus et al. 2018b). The fact that sowing experiments in other regions such as SE Brazil, NW Spain and Central Chile, resulted in germination (Calviño-Cancela & Rubido-Bará 2013; da Silva et al. 2011) is a good indicator that eucalypt seeds may escape predation. On the other hand, young eucalypt wildlings may be subject to herbivory by local species such as leaf-cutting ants (da Silva et al. 2011; Miolaro et al. 2017) and rabbits (Becerra & Bustamante 2008).

6.8 Management insights

In the introduced range, the occurrence of naturally established eucalypts, either inside or outside plantations, is normally associated with undesirable impacts. It can affect plantation productivity, aggravate fire hazard, change ecosystem services (e.g. water supply), change ecological processes or impact biodiversity, especially in heavily invaded areas. In some countries, many resources have been devoted to control eucalypts (Andreu et al. 2009; van Wilgen et al. 2012). Eucalypts are associated to both benefits and problems, being the source of much contention between different stakeholders. However, limiting natural regeneration should be a goal common to most stakeholders, so that the involvement of all stakeholders should be possible and advisable to improve the efficiency of management efforts (Novoa et al. 2018).

A rapid intervention is recommended since the control of eucalypts is relatively easy at the early stages of invasion when compared to later stages. Young plants can be easily pulled together with roots, in contrast to larger plants, especially lignotuber sprouters, which require heavy machinery or the use of chemicals on the stumps (Dana et al. 2004). Unlike other problematic invaders, such as *Acacia* spp., eucalypts do not form long-living seed banks. Seeds from most species lack dormancy (Musengi & Archibald 2017; Ruthrof 2004) and are extremely sensitive to burying, fungal attack, fire or predation (Battaglia 1996; Santos et al. 2015). As a result, most seeds should germinate within one year after shedding, and a complete eradication should be possible in a relatively short term. The limited dispersal ability in most cases, assures that surveillance and management are restricted to small areas.

The risk of eucalypt invasion varies along time and space. Species and plantations that today seem harmless may require attention in the future (Richardson & Thuiller 2007). Habitats that seem resistant to invasion may not be in the future. Invasion is more likely in unmanaged and degraded areas (Callaham Jr et al. 2013; Vaz et al. 2019). Episodic disturbances on vegetation can create opportunities for recruitment (Fernandes et al. 2018; Kirkpatrick 1977). Burnt areas require special attention and should be monitored up to one year after fire, when most seeds either germinated or have been destroyed (Santos et al. 2015).

Management of eucalypt plantations may have unexpected results. A recent study showed that harrowing, for instance, can disable most seeds on the floor, but may originate optimal micro-topographic conditions for posterior recruitment (Nereu et al. in press). Other studies suggest that coppicing may promote eucalypt recruitment (Águas et al. 2017; Deus et al. 2016), similarly to post-fire salvage logging (Águas et al. 2014), probably due to the release of canopy see banks. Post-fire management may favour the establishment of other invasive plant species (Moreira et al. 2013). On the other hand, absent management of plantations may allow a rapid accumulation of fuel of high flammability and the possibility to originate a positive feedback loop where fire occurrence leads to a forest composition of increased fire hazard (Pauchard et al. 2008; Silva et al. 2011). Burnt eucalypt stands may provide a good opportunity for forest conversion but well-informed, continuing human intervention is required (Moreira et al. 2013).

Cleared margins around plantations are likely to prevent most of eucalypt recruitment outside plantations, since most seeds shed in the first few meters (Calviño-Cancela & Rubido-Bará 2013), but there are consistent reports that eucalypts can escape far beyond narrow safety belts into adjacent habitats (Callaham Jr et al. 2013; Deus et al. 2019; Larcombe et al. 2013; Musengi & Archibald 2017). Additionally, it seems that flowering of new recruits may be favoured outside plantations (Deus et al. 2019) and that some species may acquire a short juvenile period (Deus et al. 2019; Musengi & Archibald 2017). Therefore, risk assessment should also consider the potential for long distance dispersal events.

There are solid evidences that plantations established in steep slopes represent an additional risk. First, from a geometrical perspective the distance of seed dispersal is enhanced in steep slopes. Second, steep slopes may feature reduced plant competition (Catry et al. 2015). Third, it was found that drainage lines can significantly improve the distance of naturally established eucalypts from plantations (Deus et al. 2019; Larcombe et al. 2013). Perennial watercourses revealed to be an important vector for seed dispersal and establishment of some eucalypt species in South Africa, most notably *E. camaldulensis*, which originated heavily invaded areas, radically 'transforming' riparian habitats (Forsyth et al. 2004; Henderson 1991a, 1998). It may thus be advisable removing or restricting eucalypt establishment near water courses.

The risk of invasion from eucalypt plantations is not static along time. Studies suggest that as plantations get older offspring abundance tends to increase (Águas et al. 2017; Callaham Jr et al. 2013; Larcombe et al. 2013). Seed orchards seem to pose an additional risk (Callaham Jr et al. 2013; Miolaro et al. 2017). Propagule pressure seems to play a key role as some studies found a direct correspondence between fruit abundance on planted trees and offspring occurrence (Deus et al. 2019; Larcombe et al. 2013).

Awareness is needed about the possibility of species that currently seem harmful will behave invasive in the future (Rouget et al. 2016). Ritter and Yost (2009) provides the example of *E. conferruminata* that became invasive in California after a latency period of more than 50 years. The lag-phase frequently seen in exotic species can have different reasons. For instance, the introduction of new genetic material by means of new eucalypt plantations can allow new genetic recombination with the pre-existing populations and the arise of new phenotypic and physiological traits that enhance invasiveness (Lockwood et al. 2005). Likewise, invasion may depend on the establishment of new mutualisms in the introduced, like the cases of animal-mediated pollination and seed dispersal (Richardson et al. 2000a). Risk of natural hybridisation can be assessed through flowering levels and the existence of compatible species nearby (Barbour et al. 2008).

The invasive potential of eucalypt species seems to be extremely context-specific, depending on a combination of species' intrinsic and extrinsic factors that may vary along time and between sites. Current knowledge allows to identify the most problematic species and to flag the most hazardous situations. Monitoring is necessary to record any behavioural changes along time, especially following climatic changes, that can influence not only eucalypt invasiveness but also habitat invasibility.

6.9 References

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SECTION III: General Discussion

1 General Discussion

Eucalypt plantations expanded greatly in many countries along the last decades. As plantations increased, so did concerns about their ecological impacts, including their invasive potential. Many studies have been devoted to these topics, but they are still subject to much controversy and opposing viewpoints within academics, scientists and general population. Some researchers try to help understanding phenomena in order to assist informed decisions aimed at conciliating the best interests of society. That is the spirit of the research works presented in this thesis.

With the exception of a worldwide literature review covering all eucalypt species, all studies in this thesis focus on *Eucalyptus globulus* Labill. The main reason is the predominance of *E. globulus* in the Iberian Peninsula (Iberia), particularly in Portugal, where these studies were developed. Nevertheless, *E. globulus* is one of the most widely distributed eucalypt species and, has shown in the literature review, it has been the focus of many studies investigating natural establishment. Moreover, among the few eucalypt species regarded as invasive in a worldwide review of national and international databases, *E. globulus* was the species flagged as invasive in the greatest number of regions of the world (Rejmánek & Richardson 2013).

In this thesis, we tried to provide a comprehensive perspective of *E. globulus* plantations and the processes influencing natural establishment, by encompassing a wide range of subjects, from the future dynamics of eucalypt plantations in the Iberian Peninsula into the small-scale patterns of animals foraging eucalypt seeds. The diversity of subjects addressed in this thesis is well expressed by the variety of journals where the studies were published (*Journal for Nature Conservation*; *Environmental Monitoring and Assessment*; *Biological Invasions*; *Forest Ecology and Management*; *Web Ecology*).

We should stress that, the purpose of this thesis and the studies composing it, is not to support or contradict the invasive status of eucalypts, in particular *E. globulus*. We should acknowledge that ‘populations, and not species, invade’ (Richardson & Pyšek 2012, p. 384). Despite the species’ invasiveness, i.e. the traits that enable a species to invade (Marco et al. 2002), invasion only occurs when the environmental requirements are met (Richardson & Pyšek 2006). It is thus important to understand how common natural and anthropic factors affect *E. globulus* establishment.

Given the socio-economic importance of *E. globulus* in the Iberian Peninsula, we believe that the first study (Chapter 1), projecting possible future distributions of plantations in Iberia, is of interest to a wide audience, including politicians, land managers and ecologists. This study brings awareness to the need of anticipating range dynamics of *E. globulus* plantations because they may jeopardise conservation goals, apart from current impacts derived from major landscape transformations that occurred in some Iberian regions. There is, however, another important question not addressed in that study which is worthy of discussion - how climate change may influence the invasive potential of *E. globulus*?

A study, currently under preparation for publication (co-authored by the current author), may help answering this question. One of its objectives is to project, using species distribution models, the probability of *E. globulus* recruitment across Iberia, partly using data from the Chapters 1 and 2 of the current thesis. The results suggest that areas where *E. globulus* recruitment is more likely, correspond to areas where plantation suitability is also higher. In other words, the species' invasive potential is higher in the areas where, due to higher suitability, plantations are more likely to be abundant. Other previous studies also found a positive relation between region productivity and the recruitment potential of *E. globulus* (Águas et al. 2014; Fernandes et al. 2018). Since future projections indicate a general improvement of plantation suitability in the receding range, we hypothesise that climate change may enhance the natural establishment of *E. globulus* in some regions. Another important aspect derived from climate change is the possible increase in fire danger, particularly in Portugal and Spain (Amatulli et al. 2013). As a result, the invasive potential of *E. globulus*, as well as other exotic plant species such as *Acacia* spp., may be enhanced in the future.

In the second study (Chapter 2), we performed, using Google Street View (GSV), a countrywide survey to estimate the abundance of *E. globulus* wildlings established on roadsides adjacent to plantations (Fig. 1). In this study, in order to assess the effectiveness of GSV, we replicated car surveys previously performed in another study (Catry et al. 2015). Additionally, we identified the main regional- and local-scale variables influencing offspring abundance. However, we did not spend much efforts discussing the influence of regional variables on natural establishment, because they were discussed in the analogous study using car surveys (co-authored by the author of this thesis; Catry et al. 2015). In fact, as stated in our study, 'both methods [car surveys and GSV] produced similar models of plant abundance, selecting the same explanatory variables, in the same hierarchical order of importance and depicting a similar influence on plant abundance’. Therefore, discussing the same results would be redundant.

This study showed the potential of GSV for collecting more variables (local variables) when compared to surveys in a moving car, which revealed to be important not only to understand the species’ ecology but also method-related handicaps. In this study, regional variables revealed to be more important than local-scale variables at explaining the abundance of *E. globulus* wildlings, but a posterior study, conducted across Portuguese regions, concluded that local factors were predominant (Fernandes et al. 2018). There may be several reasons explaining this difference, but we find two apparently obvious explanations. First, our sampling areas covered a wider range of environmental conditions, including areas where *E. globulus* plantations are scarcer. Second, the study conducted by Fernandes et al. (2018) probably surveyed a wider range of local conditions when compared to roadsides, including areas with different levels of disturbance.

Fig. 1 - Example of roadsides surveyed using Google Street View in the second study, showing the natural establishment of *Eucalyptus globulus* on roadsides adjacent to plantations. Note that all plants are resprouting, probably following roadside management operations

The third study (Chapter 3) provides important insights about the factors influencing the presence, abundance, development and spread of *E. globulus* wildlings. This study is of particular relevance for the topics of eucalypt naturalisation and invasion. As shown in Chapter 6, there are few studies recording and investigating the development of eucalypt wildlings, especially concerning the onset of sexual maturity. This study provides solid evidence that *E. globulus* wildlings can overcome, in great numbers, the most critical stages of survival, and that they reach reproductive maturity, including adult wildlings outside plantations up to considerable distances from mother-plants (Fig. 2). Hopefully, these findings, corroborated in a smaller degree by another study conducted in Portugal (Fernandes et al. 2016), will bring awareness about the invasive potential of the species and the factors enhancing it, and may help disproving the claims that 'all eucalypt wildlings die' and that offspring 'do not escape from plantations'.

Fig. 2 - Perspective from the inside of a *Eucalyptus globulus* plantation over two large, adult wildlings (probably) escaped from plantations. Photo taken under the frame of the third study of this thesis, authored by E. Deus / H. Matias

There is, however, the need of further research to investigate the reproductive potential of naturally established eucalypts. In other words, it is worth exploring if eucalypt wildlings produce viable seeds capable of promoting further encroachment and spread of the species. An ongoing project, with the participation of the author of the current thesis, tries to answer this and other questions. Using satellite imagery and genetic analyses, project WildGum II is trying to establish parental relations between plantations and natural populations of *E. globulus*.

One of the most interesting findings in the third study (Chapter 3), was the existence of very small reproductive wildlings, suggesting that the species may have a short juvenile period, one important trait normally associated to invasive plants (Grotkopp et al. 2002; Rejmánek & Richardson 1996). It is known that, in *E. globulus*, early flowering is under strong genetic control (Jordan et al. 1999). Given the intensive breeding programs in Portugal (Borralho et al. 2007; Borralho et al. 1993; Greaves et al. 2004), and elsewhere for other eucalypt species (Harwood 2014; Potts 2004), it would be important to know how these programs aimed at enhancing plant performance and resistance can, as a side effect, enhance the species' invasiveness.

Eucalypt plantations and their surroundings are normally subject to intensive management, except, obviously, abandoned lands, which have become increasingly common in Portugal. Research on eucalypt management is mostly directed to enhance plantation performance and resistance (e.g. Carneiro et al. 2008; Gonçalves et al. 2008; Vargas et al. 2017; Wingfield et al. 2013) and, to a small degree, to mitigate ecological impacts by, for instance, improving biodiversity (e.g. Amazonas et al. 2018; Brockerhoff et al. 2013; Carneiro et al. 2007; Fabião et al. 2002). The fourth study (Chapter 4), brings awareness to the possible impacts of standard management operations on eucalypt recruitment, a subject that, surprisingly, has been poorly explored. Given the biological and ecological similarity between eucalypt species, and the similar procedures used in plantations and their surroundings across different countries, the findings of this study can be useful for other regions and eucalypt species.

The fifth study (Chapter 5) provides novel insights into the possible role of local fauna on eucalypt recruitment. Given the primordial importance of ants limiting eucalypt recruitment in the native range (Andersen 1982; Ashton 1979; Wellington & Noble 1985), we were surprised by the little knowledge about animal-seed interaction in the introduced range. Our experiment showed that *E. globulus* seeds were highly attractive to local fauna, even though some locations acted as ‘safe zones’ for seeds due to reduced foraging. These results suggest that animal-seed interaction, which may include both seed predation and seed dispersal, with contrasting implications, may play an important role in eucalypt recruitment in the introduced range. As a result, this study encourages further research on this topic. For instance, there may be seasonal variations in seed predation, similarly to observations in the native range, where seed predation was higher in summer (Andersen & Ashton 1985; Ashton 1979). Also, it would be opportune to assess the effect of disturbances such as management or wildfires on seed-harvesting animals, and how it may affect seed predation.

Another interesting aspect in this study (Chapter 5), which was not properly discussed in the paper, is that *E. globulus* seeds were made available in locations where the species did not exist, i.e. local fauna never had contact with eucalypt species. However, *E. globulus* seeds were immediately perceived as a food source, and an interaction between species was rapidly established, with direct consequences for the natural establishment of *E. globulus*. The animal interaction with this exotic species supports the assumption of the ecological integration of eucalypts, under the broader concept of ‘novel ecosystems’, where, through human agency or environmental change, new combination of species arise, with the potential for changes in ecosystem functioning (Hobbs et al. 2006; Seastedt et al. 2008). Examples of mutualistic relations, with direct implications for the reproduction and natural establishment of *E. globulus*, is the pollination of *E. globulus* by native bird species (Calviño-Cancela & Neumann 2015) and the establishment of novel associations with ectomycorrhizal fungi (Santolamazza-Carbone et al. 2019).

The final study (Chapter 6) provides a comprehensive review and overview on the literature tackling the invasive potential of eucalypts in the introduced range. The literature survey was targeted at all eucalypt species from the genera *Eucalyptus*, *Corymbia* and *Angophora*, even though most studies addressed *Eucalyptus* spp., and especially *E. globulus*, which was the subject of more and the most detailed studies, partly due to the studies presented in this thesis. This study represents the culmination of the work developed by the current author along the last years and shows how the studies developed in the frame of this thesis contribute to the existing knowledge, by providing new empirical insights related to the natural establishment of *E. globulus* in the introduced range.

In this review, there is a special focus on the species *E. globulus* in Portugal. The first reason is that *E. globulus* is the main focus of this thesis. Second, *E. globulus* is the most investigated species regarding natural establishment outside the native range, featuring studies in six different countries, which is a good indicator of the importance of this topic. Third, most of these studies were conducted in Portugal. We should also note that this is the third PhD. thesis focusing on the natural establishment of *E. globulus* in Portugal (the other two are: Águas 2019; Fernandes 2016). Finally, Portugal is, in fact, a very particular case, where we can find a particular combination of factors favouring the natural establishment and the invasive behaviour of *E. globulus*, such as: the climatic suitability for the species (Catry et al. 2015; Deus et al. 2018); the predominance of small, private land plots, particularly in Central and North Portugal (Beires et al. 2013); a private-propelled proliferation of small *E. globulus* plantations, enough to completely transform regional landscapes; the mismanagement or abandonment of *E. globulus* plantations (see Fig. 3-5), especially after fires (Silva et al. 2011; Vaz et al. 2019); and the recurrent wildfires in the regions where *E. globulus* plantations are widespread (Nunes et al. 2016).

Fig. 3 - Abandoned *E. globulus* plantations in Portugal, probably affected by wildfire in its recent history, with high fuel loadings and no safety margins relatively to the road. This is a common sight in marginal roads across Central and North Portugal. Photo retrieved from Google Earth

Fig. 4 - Perspective of the inside of mismanaged *Eucalyptus globulus* plantations in Portugal. We assume they are not abandoned because at that time they were ‘managed’ by pulp companies. Photos by H. Matias

Fig. 5 - An *Eucalyptus globulus* plantation in Portugal managed by pulp industries. Photo by H. Matias

In Portugal, two months after a tragic wildfire where dozens of people perished trapped in their cars between two burning eucalypt plantations (Comissão Técnica Independente 2017), a law was published aiming to restrict and limit the expansion of eucalypt plantations (Lei n.º 77/2017 de 17 de Agosto 2017), while trying to promote the conversion of eucalypt plantations into eco-friendlier land uses, such as native forests or agriculture areas. This goal will be hard to achieve for many reasons: 1) most land in Portugal is privately owned, with a predominance of small land plots in the regions where eucalypts are most spread; 2) property rights in Portugal are sacredly protected; 3) conversion of eucalypt plantations takes a lot of resources; 4) there are few precedents in Portugal of ecological restoration.

Conversion of eucalypt plantations to native vegetation was accomplished in the neighbouring Spanish province of Andalucía, but it was possible due to different conditions, such as the location of plantations in public lands and a higher investment in ecological restoration and control of invasive plants. In Andalucía, eucalypt plantations also expanded greatly since the mid-20th century up to the late 1980's, reaching c. 270,000 ha, both in public and private lands. However, in the 1990's, severe restrictions were applied to the cultivation of eucalypts and a plan was implemented to restore eucalypt plantations in public lands. Two decades later, with much efforts, more than 100,000 ha of eucalypt plantations were converted into mixed stands of native species, pastures, agricultural areas and shrublands (Álvarez 2011). Such an enterprise in Portugal would be hard to achieve for the reasons pointed above. In contrast, in Spain, much resources are devoted to control eucalypts (Andreu et al. 2009).

Eucalypts are not officially classified as invasive, neither in Portugal nor in Spain, despite figuring in lists of invasive plants defined by experts in both countries (Dana et al. 2004; Marchante et al. 2014). It is widely acknowledged that *E. globulus* is a less aggressive invader when compared to other woody alien plants such as *Acacia* spp., *Ailanthus altissima* (Mill.) Swingle or *Hakea sericea* Schrader. In Portugal, Fernandes et al. (2016) concluded that the native *Pinus Pinaster* Aiton was a more successful invader than *E. globulus*. It would be senseless to apply to *E. globulus* the same legal restrictions as other invasive plants, which would include to forbid their cultivation. It should be acknowledged the great socio-economic importance of *E. globulus* in some Iberian regions. On the other hand, we should also acknowledge the potential regional and local impacts of eucalypt plantations. It seems clear the need of implementing effective regulation towards landscape planning and active management of plantations, aimed at mitigating a wide array of environmental impacts, such as on biodiversity, water resources, wildfires and invasion of vulnerable habitats (e.g. Abelho & Graça 1996; Barrocas et al. 1998; Calviño-Cancela et al. 2012; De la Hera et al. 2013; Rodríguez-Suárez et al. 2011; Souto et al. 2001). The unsuccessful regulation was demonstrated in the first study (Chapter 1), revealing that plantations of *E. globulus* expanded inside protected areas of the Natura 2000.

Currently, due to a legislative gap, the escape of eucalypts from plantations (or other invasive plants) into adjacent lands becomes the problem of someone else, as a part of a ‘natural process’. In other words, there is no accountability due to the mismanagement of plantations. A well-known case is ‘Tapada de Mafra’, a natural area extending over 1,000 ha, delimited in the 18th century at the time of the construction of its National Palace, for the leisure of the royal family. According to the records of this property, eucalypts were never planted inside it. On the other hand, plantations proliferated in its surroundings. Along the last years, there have been many invasion foci by *E. globulus* inside the Tapada de Mafra, some extending hundreds of meters away from the nearest plantations. As a result, over the years, many resources were devoted to control the invasion, without any accountability by the owners of the surrounding plantations, which include plantations that seem to be abandoned (Fig. 6).

Fig. 6 - Invasion of the Tapada de Mafra in Portugal by *E. globulus* (behind the wall on the right side) originated by adjacent plantations (on the left). Image retrieved from Google Street View. Coordinates (decimal degrees): 38.942274º N; 9.286283º W

The studies presented in this thesis make part of a recent effort to understand the natural establishment of *E. globulus*, which include some other studies published in the last five years (e.g. Águas et al. 2014; Águas et al. 2017; Calviño-Cancela & Rubido-Bará 2013; Catry et al. 2015; Fernandes et al. 2016). Also, some works and projects on this subject are currently ongoing, such as the WildGum II project. These studies are thus aimed at complementing the existing knowledge and at helping to identify the locations, situations and periods where natural establishment is enhanced, in order to help preventing or mitigating the negative ecological impacts from plantations.

2 Conclusions

In some Iberian regions, plantations of *Eucalyptus globulus* Labill. expanded exponentially since the mid-20th century. Like many eucalypts in other regions of the world, in Iberia, *E. globulus* has been subject to much controversy regarding its ecological impacts and invasive potential. Despite an increasing number of studies in the last years, there are still poorly explored topics, some of which we tried to tackle in six studies, each corresponding to one chapter in the Section II of this thesis.

In Chapter 1, we stressed the need of anticipating future dynamics following climatic changes. We estimated that the suitable range of *E. globulus* plantations in Iberia will probably shrink while conflicts with high biodiversity areas are likely to increase. In Chapter 2, a countrywide survey in Portugal, conducted using Google Street View (GSV), revealed the extraordinary capacity of *E. globulus* to successfully reproduce across a wide range of environmental conditions, even though offspring abundance was strongly influenced by climatic variables. Despite less influential, local variables, collected exclusively with GSV, revealed to be relevant to help understand the species' ecology and method-related limitations. GSV was also proven to be a cost-effective alternative to car surveys to study alien invasive plants. In Chapter 3, surveys along the edges and outside *E. globulus* plantations provided strong evidences that the species is naturalised and can successfully invade adjacent habitats, since most wildlings reached adulthood and some possessed reproductive organs. Factors such as soil cover, plantation age and the number of capsules on nearby trees helped explaining the presence and abundance of *E. globulus* wildlings. We found wildlings spread up to 75 m from plantations, and the distance of dispersal was influenced by the presence of drainage lines and the direction of dominant winds. This thesis explored other factors that can play a key role at the early stages of recruitment, namely the execution of standard management operations (Chapter 4) and animal interaction with *E. globulus* seeds (Chapter 5). In Chapter 4, we found that, in general, standard management operations, including prescribed fire, soil stripping and soil harrowing, may limit severely the germination from pre-existing seeds in the soil, but operations such as harrowing may favour germination and survival from seeds shed after this treatment. In Chapter 5, we confirmed that animals harvest seeds from *E. globulus*, which revealed to be very appealing to local fauna, and that seed harvesting animals, through seed predation or dispersal, can help explaining the spatial heterogeneous recruitment patterns frequently recorded in other studies. In Chapter 6, a literature review showed that an increasing number of studies have been investigating the natural establishment of eucalypts. Factors enhancing eucalypt recruitment are common to many eucalypt species, and include higher propagule pressure, reduced plant competition and the occurrence of disturbances. *Eucalyptus globulus* has been the focus of more and the most detailed studies, particularly in Portugal.

Portugal seems to feature a particularly favourable combination of factors enhancing the naturalisation and invasion by *E. globulus*. Current policies in Portugal do not seem sufficient or adequate to address ecological impacts from *E. globulus* plantations. The studies presented in this thesis may help understanding the ecology of *E. globulus* and the processes affecting natural establishment, in order to help preventing or mitigating noxious impacts from plantations.

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