UNIVERSIDADE DE LISBOA

FACULDADE DE CIÊNCIAS

Natural regeneration and expansion capacity of forestry species: *Pinus pinaster* and *Eucalyptus globulus*

Doutoramento em Biologia
Especialidade em Ecologia

Patrícia Alexandra Tinoco Fernandes

Tese orientada por:
Professora Doutora Otília Correia
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Documento especialmente elaborado para a obtenção do grau de doutor

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Esta dissertação teve o apoio da Fundação para a Ciência e a Tecnologia (FCT) e da The Navigator Company através da bolsa de doutoramento em empresa SFRH/BDE/51709/2011

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Apoio financeiro

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Nota Prévia

A presente tese apresenta resultados de trabalhos já publicados ou em preparação para publicação (capítulos 2 a 5), de acordo com o previsto no nº2 do artigo 25º do Regulamento de Estudos Pós-Graduados da Universidade de Lisboa, publicados no Diário da Republica II série nº 57 de 23 de março de 2015. A autora da dissertação declara que esta dissertação é resultado do seu próprio trabalho. Tendo os trabalhos sido realizados em colaboração, a autora esclarece que participou integralmente na concepção de todos os trabalhos apresentados, incluindo o desenvolvimento de métodos, a obtenção dos dados, a análise e discussão dos resultados, bem como na redacção de todos os manuscritos. A presente tese contém ilustrações científicas da autoria de Catarina Costa, Francesco Milanese e José Perico.

Lisboa, Dezembro de 2016

Patrícia Alexandra Tinoco Fernandes

Ao João

À Diana

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ABSTRACT

Nowadays, the expansion of forestry trees into habitats outside plantations is a relevant concern for managers and conservationists, and a relevant issue for biological invasion research. Among the most planted forest species worldwide, we can find Eucalyptus and Pinus species. Specifically, Portuguese planted forests are predominantly composed of two species, *Eucalyptus globulus* and *Pinus pinaster*. However, little is known about the regeneration and spread capacity of these species under different habitats and environmental conditions. In Portugal, given their exotic origin and their widespread use in forestry*, E. globulus* has been a controversial species mainly in relation to the presence or not of the invasive behavior. Thus the overall goal of this thesis was to identify the spatial recruitment patterns of *P. pinaster* and *E. globulus* into habitats surrounding the plantations analysed according the specific objectives: (i) to determine the capacity of these species to colonize outside the planted areas, (ii) to evaluate their seedling establishment capacity under different ecological conditions, (iii) to identify those conditions that favor or hamper these species establishment, and (iv) to understand the main factors that drive natural establishment of these species. During this study, all these aspects were adressed, and for the first time, a country wide detection survey in the natural vegetation adjacent to plantations was conducted, recording *P. pinaster* or *E. globulus* seedlings establishment by natural regeneration. In addition, seedling emergence and survival, were investigated during two years using a seed addition experiment through a manipulative study under natural conditions.

These species presented very different recruitment characteristics, regarding the amount of established seedlings and the distance of colonization, much higher for *P. pinaster*. In mature mixed forests, natural recruitment of *P. pinaster* was also significantly higher than *E. globulus*, even when *P. pinaster* was not a dominant planted species. Considering the seedling survival capacity, *E. globulus* displayed higher mortality rate during initial phase of seedling growth, when compared to *P. pinaster*. The better climate predictors explaining *E. globulus* recruitment were temperature seasonality and annual precipitation. *E. globulus* was more likely to be present in areas with low temperature seasonality and high annual precipitation. In addition, seedling survival experiment showed also that water deficit can compromise summer survival of *E. globulus* seedlings. On the other hand, it was not clear how *P. pinaster* recruitment is affected by climatic conditions. Based on the models explored in this study, no climate variable explained *P. Pinaster* recruitment. Recruitment models reveled that plantation characteristics (age and area) had no significant influence on *P. pinaster* and *E. globulus* recruitment. However, in the unmanaged mature plantations (older than 25 years and left unharvested after optimal age) a notoriously higher recruitment of these species than in the industrial plantations was found. Finally, site characteristics (such as habitat type, vegetation cover, and disturbance) were the most important group of variables influencing *P. pinaster* and *E. globulus*. Forest was the most resistant habitat to these species establishment. In the case of *E. globulus* also grasslands were very resistant to their establishment. Results showed that vegetation cover alone explained more than 47% of eucalypts occurrence variability and a negative association was found between vegetation cover and eucalypt occurrence and density. The same pattern was found in relation to vegetation disturbance level, which was the most important factor explaining eucalypt density. The high importance of site characteristics in this study reflects that most of the variability in these species establishment occurs at small spatial scales.

In conclusion, the results that support this thesis suggest that the colonization outside plantation by *E. globulus* will likely be remarkably slow when compared to *P. pinaster* (species with long-distance seed dispersal and higher seedling survival capacity). Moreover, localized recruitment and very low levels of establishment, and the success of native species recruitment under eucalypt forests suggested that currently, *E. globulus* does not demonstrate an invasive behavior in adjacent areas to the plantations, irrespectively to environmental land use characteristics. Indeed, *E. globulus* limited expansion ability seems to be related with the combination of two characteristics: limited seed dispersal; and high seedlings mortality rate. In contrast, *P. pinaster* seedlings had high plasticity and drought resistance.

The intensive management of these plantations (mainly *E. globulus*) might contribute to diminish their ability to develop a significant propagule pool. However, mature plantations (left unharvested) required an increased attention, as the propagule pressure builds with time. Other important finding of the present study was that the small-scale factors (vegetation cover, disturbance and habitat type) were more important than the others high-scale factors (Climate and plantation characteristics) and they are essential to precise predictions at more local scales. Taken into account this thesis results, it was suggested the establishment of "sentinel sites" using in-situ visits during conventional management as described here. This monitoring system could, over time, produce very valuable data to improve our understanding of forestry species colonization dynamics and could also alert managers to situations where some form of management intervention is needed.

The findings presented here were crucial to understand the spatial pattern of *P. pinaster* and *E. globulus* natural recruitment, and the spread capacity of these important forestry species through their response in early colonization stages to environmental conditions, and can help to explain future shifts in these species distribution and natural establishment outside the planted areas.

KEYWORDS: forestry plantations; invasion risk; natural regeneration; seedling survival; forest management.

RESUMO

Durante as últimas décadas, a crescente procura mundial de produtos florestais (tais como madeira, painéis, biocombustível e papel) e a necessidade para restaurar grandes áreas degradadas, levou a um aumento global da área de floresta plantada. As espécies de *Eucaliptus* spp. e *Pinus* spp. estão entre as mais plantadas em todo o mundo. A utilização destas espécies em plantações florestais está essencialmente relacionada com a elevada plasticidade ecológica destas espécies (conseguem tolerar uma ampla gama de condições ecológicas), com o rápido crescimento e a elevada produtividade. Assim, estas espécies florestais têm um papel significativo na economia regional e nacional, através de vários produtos e serviços do ecossistema. No entanto, a elevada produtividade destas espécies e a sua plantação extensiva têm levantado algumas preocupações relacionadas com a eventual capacidade destas espécies invadirem outros habitats. O risco de colonização de habitats naturais por espécies florestais tem sido, nos últimos anos, uma preocupação quer para gestores florestais quer para conservacionistas. Neste sentido, é essencial avaliar a capacidade de colonização das espécies florestais plantadas e compreender os principais fatores que influenciam o seu estabelecimento natural.

No caso de Portugal, a floresta é maioritariamente plantada, sendo as espécies *Eucalyptus globulus* e *Pinus pinaster* as mais plantadas para a obtenção fibra e de madeira, respetivamente. *E. globulus*, por ser a espécie exótica mais plantada em Portugal, tem sido uma espécie controversa, mais recentemente em relação à presença ou não de comportamento invasor. No entanto, e apesar da grande presença destas espécies em território nacional, muito pouco se sabe realmente sobre a sua capacidade de regeneração natural e capacidade de expansão das suas populações. Por exemplo, *E. globulus* aparece classificado como espécie invasora no guia de campo de Marchante et al. (2014) sem qualquer avaliação quantitativa do seu estabelecimento natural. Além disso, é de notar que existe alguma confusão em torno do conceito de espécie invasora, sendo este muitas vezes mal aplicado. Tem sido frequente ver associado o termo “invasor” às espécies exóticas plantadas não devido ao seu comportamento invasor mas sim devido à extensão da área plantada. Assim é importante esclarecer que entendemos como espécie invasora a espécie exótica que produz descendentes férteis frequentemente em grande quantidade e os dispersam a distâncias consideráveis da planta-mãe possuindo assim um enorme

potencial de se espalhar numa grande área, sem intervenção directa do Homem, e com potencial para ocupar áreas extensas, em habitats naturais ou semi-naturais. Desta forma, o presente estudo teve como principal objectivo avaliar a regeneração espontânea e a capacidade de expansão de *E. globulus* e *P. pinaster* a partir das suas plantações para os habitats naturas ou semi-naturais adjacentes às mesmas. Foi avaliado o papel das condições climáticas, pressão de propágulos e características do habitat (tipo de habitat, tipo de solo, grau de perturbação e a cobertura da vegetação) no estabelecimento espontâneo de *E. globulus* e *P. pinaster*, identificando os principais fatores que determinam o sucesso destas duas espécies florestais.

Esta tese é constituída por seis capítulos. O primeiro capítulo corresponde à introdução geral do trabalho, os quartro capítulos seguintes correspondem a artigos científicos publicados ou submetidos em revistas internacionais de arbitragem científica indexadas no *Science Citation Index*, terminando com o capítulo de discussão e conclusões finais.

No capítulo 1, introdução geral, é apresentado um enquadramento do tema da presente tese, onde são abordados os principais fatores responsáveis pelo sucesso no estabelecimento natural das plantas e que determinam a sua capacidade de colonização de novas áreas. Foi abordado o enquadramento destas duas espécies em Portugal, as suas principais características, bem como o estado da arte relativamente à avaliação do potencial invasor destas duas espécies.

No capítulo 2 foi identificado o padrão espacial do estabelecimento natural de *P. pinaster* e de *E. globulus* nos habitats naturais envolventes às plantações. Foram também analisados os principais fatores (clima, tipo de habitat, e área da plantação) que afetaram o padrão espacial de colonização destas duas espécies. O método de amostragem consistiu no registo de individuos de *E. globulus* e *P. pinaster* estabelecidos por regeneração natural em vários habitats adjacentes às plantações destas duas espécies. Foram estabelecidos transectos num gradiente de distância a partir do limite da plantação até aos 105 metros. No total, foram realizados 256 transectos ao longo de 15 plantações de *P. pinaster* e 749 transectos ao longo de 23 plantações de *E. globulus*, na região norte e centro de Portugal. As duas espécies apresentaram um padrão de expansão natural semelhante verificando-se uma diminuição exponencial em função da distância ao limite da plantação. No entanto, os resultados mostram que estas duas espécies apresentam uma capacidade de estabelecimento e expansão muito diferentes. O estabelecimento natural de *P. pinaster* é caracterizado por uma elevada densidade e com capacidade de alcançar distâncias superiores a 100 m da plantação. Em contraste, o estabelecimento natural de *E. globulus* fora das áreas plantadas foi esporádico e essencialmente junto às plantações. As variáveis climáticas que melhor explicaram o estabelecimento de *E. globulus* foram a variação sazonal de temperatura e a precipitação anual. A probabilidade de estabelecimento de *E. globulus* é maior em áreas de menor variação sazonal de temperatura e de elevada precipitação. Por outro lado, não ficou claro de que forma as variáveis climáticas influenciam o estabelecimento natural de *P. pinaster*. Com base nos modelos explorados neste estudo, nenhuma variável climática foi significativa na explicação do estabelecimento natural de *P. pinaster*. A floresta foi o tipo de habitat mais resistente ao estabelecimento destas duas espécies florestais. No caso de *E. globulus* as áreas de vegetação herbácea foram também muito resistentes ao seu estabelecimento. Em conclusão, o estabelecimento localizado e esporádico de *E. globulus,* e o sucesso da regeneração de espécies nativas (incluindo *P. pinater*) nas plantações de eucalipto, indicam que a espécie *E. globulus* não apresenta um comportamento invasor nos habitats naturais adjacentes às plantações, independentemente do tipo de habitat ou das condições ambientais consideradas.

No capítulo 3 foram identificados os principais fatores que determinam a presença e a densidade de *E. globulus* por regeneração natural em diferentes escalas espaciais, e a sua importância relativa. Recorrendo a um método analítico da macro-ecologia, análise Multimodal, foi possível identificar os melhores modelos explicativos para a ocorrência e densidade de *E. globulus*. Todas as variáveis explicativas foram classificadas de acordo com a sua importância na regeneração natural do eucalipto. Foram utilizados os dados já obtidos na amostragem do capítulo 2, incluindo também os dados obtidos na amostragem de plantações de *E. globulus* no sul do País. No total foram amostradas 50 plantações de *E. globulus* (1579 parcelas de amostragem) de norte a sul de Portugal continental. A presença de eucalipto foi apenas registada em 8% das parcelas amostradas. As características do local (como o tipo de habitat, cobertura vegetal, e perturbação) foram o grupo de variáveis mais importante na explicação da regeneração natural de *E. globulus*, comparativamente com as variáveis climáticas ou as características da plantação, como a sua idade e área de ocupação do terreno. A densidade da vegetação envolvente e o nível de perturbação do solo foram as variáveis mais significativas. O estabelecimento de *E. globulus* ocorreu essencialmente em áreas com baixa cobertura de vegetação e com elevada perturbação.

A elevada importância das variáveis locais relativas ao tipo de habitat (explorado no capítulo 2 e 3) e a cobertura da vegetação (explorado no capítulo 3) em relação às variáveis climáticas (exploradas nos capítulos 2 e 3) mostram que a variabilidade no estabelecimento destas duas espécies estudadas ocorre essencialmente numa escala espacial muito reduzida. Consequentemente, esses fatores de pequena escala são essenciais para previsões mais precisas do risco de colonização destas espécies. Assim, os esforços de monitorização devem concentrar-se sobre os fatores determinantes para a colonização à escala local como o tipo de habitat, cobertura da vegetação e nível de perturbação.

No capítulo 4 foi avaliada a capacidade de sobrevivência das plântulas de *P. pinaster* e *E. globulus* em diferentes condições ecológicas e identificadas as condições que favorecem ou dificultam o estabelecimento natural destas duas espécies. Especificamente, foram avaliados os efeitos da perturbação (remoção de vegetação), tipo de habitat (floresta e matagal) e clima (norte e sul de Portugal) na germinação e sobrevivência das plântulas através da monitorização de sementeiras realizadas no local. Esta informação permitiu corroborar e complementar os resultados obtidos nos dois capítulos anteriores. Os resultados mostram que o efeito do *stress* hídrico, disponibilidade de luz e a presença de perturbação no estabelecimento natural destas espécies é dependente do contexto climático. Apesar de maior percentagem de germinação de sementes, *E. globulus* apresentou uma taxa de mortalidade muito elevada e significativamente superior à de *P. pinaster*. As plântulas de *P. pinaster* conseguiram estabelecer-se em ambos os locais e em ambos os habitats. Em contraste, todas as plântulas de *E. globulus* morreram, exceto nas parcelas instaladas nos matos com perturbação na zona Norte. A percentagem de sobrevivência em *E. globulus* foi muito baixa e significativamente inferior à registada para *P. pinaster* (0,27% vs 7,96%). As plântulas de *P. pinaster* apresentaram assim uma elevada plasticidade (capacidade de sobreviver em diferentes condições) e resistência à seca, comparativamente com as de *E. globulus*. Deste modo, o capítulo 4 permite explicar o número muito baixo de plântulas de *E. globulus* nas áreas envolventes às plantações, registados nos capítulos 2 e 3. Para além da limitada capacidade de dispersão de sementes, discutida no capítulo 2, esta espécie apresenta também uma elevada taxa de mortalidade e uma baixa resistência ao *stress* hídrico nas primeiras fases de crescimento das plântulas, reduzindo a colonização de novas áreas.

A amostragem nos capítulos 2 e 3 foi realizada em plantações florestais geridas (industriais). No entanto, a existência de plantações abandonadas ou sem gestão são uma realidade no nosso País, principalmente no norte e centro litoral. Assim, no capítulo 5 foi avaliada a regeneração natural de *P. pinaster* e *E. globulus* em plantações maduras (com mais de 25 anos) e sem qualquer tipo de gestão. Paralelamente, foi também contabilizado o número de espécies presentes nestas áreas de modo a avaliar o valor deste tipo de floresta como habitat para a vegetação nativa. Os resultados mostram mais uma vez que o estabelecimento natural de *P. pinaster* foi significativamente superior ao de *E. globulus*, mesmo em povoamentos em que *P. pinaster* não é a espécie plantada dominante. No entanto, em ambas as espécies, a densidade de indivíduos estabelecidos por regeneração natural registada nas plantações abandonadas foi superior à registada no capítulo 2, em plantações geridas. Assim, a gestão intensiva das plantações (essencialmente nas plantações de *E. globlulus*) pode contribuir para a diminuição de pressão de propágulos, afetando a probabilidade de estabelecimento por regeneração natural destas espécies. No entanto, no caso de plantações mais velhas e sem gestão, a pressão de propágulos será maior, aumentando a probabilidade de estabelecimento por regeneração natural destas espécies ao longo do tempo. A elevada regeneração de *Quercus spp.* (presente em 88,5 % das parcelas amostradas) bem como outras folhosas pode indicar que estes povoamentos não geridos de *P. pinaster* e *E.globulus* têm um potencial considerável para a conversão em povoamentos mistos com espécies de *Quercus spp.* e outras espécies nativas. Por outro lado, também foi registada a ocorrência de espécies invasoras, nomeadamente *Acacia spp*., em 17,3% das parcelas de amostragem.

Finalmente, no capítulo 6 são apresentadas as várias conclusões que integram os principais resultados obtidos nos capítulos anteriores, assim como as suas contribuições e implicações no contexto da regeneração e capacidade de expansão natural destas duas espécies florestais. Do ponto de vista da gestão, são também propostas algumas estratégias que podem ser integradas de forma a evitar o risco de colonização indesejada por estas espécies.

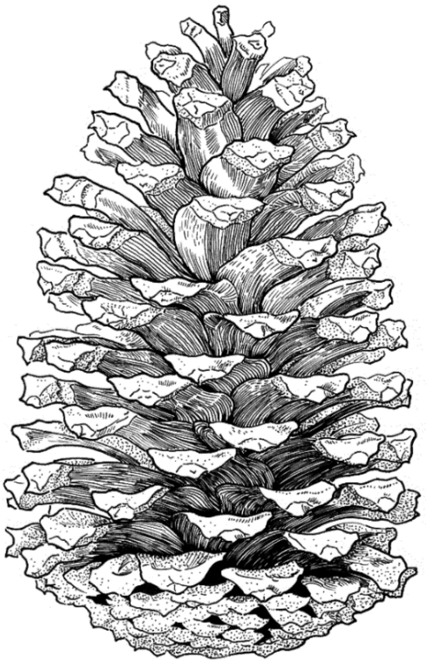
KEY-WORDS: plantações florestais; risco de invasão; regeneração natural; sobrevivência de plântulas; gestão florestal.

CHAPTER 1

GENERAL INTRODUCTION

&

GENERAL AIMS AND THESIS OUTLINE



GENERAL INTRODUCTION

Biological invasions occur when natural communities are threatened by an increase in distribution and abundance of exotic species, causing significant changes in the ecosystem composition, process and structure (Levine et al., 2003). Indeed, this phenomenon is already considered one of the main factors of global change. Nowadays, the expansion of forestry trees into habitats outside plantations is a relevant concern for managers and conservationists, and a relevant issue for biological invasion research (e.g. Dodet and Collet, 2012; Richardson and Rejmánek, 2011; Richardson, 1998; Richardson et al., 2013). In order to contribute to a sustainable forest management, minimizing ecological risks and maintaining economical productivity, it´s crucial to understand the main factors that drive forestry species natural establishment and to determine their colonization capacity outside their planted areas.

Forestry plantation and the invasion risk

During the last decades the increasing world demand for forest products (such as timber, wood, biofuel and paper) and the needs to restore large degraded areas has led to a global increase in planted forest area (FAO, 2010). Contrary to the area of natural forests, which has been declining, planted forests are expanding (FAO, 2010). In some regions, plantations comprise the major proportion of forest area (FAO, 2010). In 2010, the total area of planted forest was estimated to be 264 million hectares (7% of the total forest area worldwide; FAO, 2010), and this increases to around 278 million hectares in 2015 (FAO, 2015). Planted forests by definition comprise trees established through planting and/or through deliberate seeding of native or exotic tree species (Brundu and Richardson, 2016). In many countries, plantation forestry consist mostly on a few fast-growing species (Richardson, 1998). Among the most planted forest species worldwide, we can find *Eucalyptus* and *Pinus* species, being *Eucalyptus* the most planted tree species in the world (FAO, 2010). The use of these species in forestry plantations is primarily related to the wide ranges of ecological requirements of these species, to their rapid growth and high productivity. In many countries, these forestry species make a significant contribution to the economy and provide multiple products and ecosystem services (Brundu and Richardson, 2016). On the other hand, these special characteristics present in forestry species and their widespread use in forestry sector also impose new concerns related to the ability of these species to invade natural and semi-natural habitats surrounding plantations (Dodet and Collet, 2012; Essl et al., 2010; Richardson and Rejmánek, 2011; Richardson, 1998; Van Der Meer et al., 1999).

Invasive plant species is defined as exotic plants that produce reproductive offspring, often in very large numbers, at considerable distances from the parent plants, and thus have the potential to spread over a large area (Box 1; Pyšek et al., 2004). Thus the efficiency of dispersal, and seed germination and establishment capacity is essential to a naturalized species to become invasive (see definitions in Box 1). Indeed Wallace et al., (2008), indicates that invasion risk is highly related to the distance that seeds can travel from their point of origin (dispersion ability). Therefore, is crucial to know forestry species natural establishment and expansion capacity to better evaluate their invasive potential. Eucalypts are generally viewed as markedly less invasive than several other widely cultivated trees (e.g. pines and acacias; Rejmánek and Richardson, 2011). In this regard, not all alien species become invasive and there are several ecological factors that can prevent their passage to an invasion state (Fig. 1).

BOX 1. Key concepts (adapted from Pyšek et al., 2004)

Native plants	Taxa that have originated in a given area without human involvement or that have arrived there without intentional or unintentional intervention of humans from an area in which they are native.
Exotic plants	Plant taxa in a given area whose presence there is due to intentional or unintentional human involvement, or which have arrived there without the help of people from an area which they are alien.
Naturalized plants	Exotic plants that sustain self-replacing populations for at least 10 years without direct human intervention by recruitment from seed or ramets (tillers, tubers, bulbs, etc.) capable of independent growth. Naturalized plants do not necessarily invade natural, semi-natural or human-made ecosystems. Taxa persisting in sites where they were planted (after cultivation as ceased) represent a special category, but they can be classified within the current scheme as either casual or naturalized.
Invasive plants	Naturalized plants that produce reproductive offspring, often in very large numbers, at considerable distances from the parent plants, and thus have the potential to spread over a large area, with profound impacts on ecosystems.

Main factors associated with plant recruitment success

Natural regeneration involves many stages in the life cycle of plants, with seedling establishment recognized as a critical step for recruitment success (Castro et al., 2004; Houle, 1996; McAlpine and Jesson, 2008). Young seedlings are more vulnerable to resources availability and stress factors, which determine species establishment success and distribution (Niinemets and Valladares, 2006). Conceptual models that explain introduced plants establishment success have pointed to the combination of having enough propagule pressure and a suitable environment, biotic and abiotic conditions for establishment (Fig.1; Catford et al., 2009; Chytrý et al., 2008a). Abiotic factors are the main drivers to forestry plantations distribution. Forestry species are planted in accordance with their productivity under different edafo-climatic conditions, targeting abiotic conditions highly suitable for the species (Fig. 1). Forestry species can spread to nearby natural and semi-natural ecosystems by natural regeneration (naturalization; Fig. 1). Although the ability of species to recruit (propagule pressure), the susceptibility of the environment (or it´s opposite, ecological resistance) to the establishment of these planted species, and their capacity to spread at considerable distances from the parent tree (dispersion ability) are crucial for their invasive behavior (Fig. 1).

Among the abiotic factors that determine plant establishment, the effect of climate is probably the most studied (Thuiller et al., 2005). Climate has been considered as the main drivers of plant distribution from continental to regional scales (Milbau et al., 2009a; Pearson et al., 2004). Specifically, drought summer in the Mediterranean region is highly limiting for plant performance and recruitment (Chytrý et al., 2008b). Accordingly, one of the major limiting constrains to *E. globulus* and *P. pinaster* natural establishment in Iberian Peninsula is water availability (Águas et al., 2014; Almeida et al., 1994; Alves et al., 2012; Catry et al., 2015; Rodríguez-García et al., 2011, 2010; Ruano et al., 2009). However, there is a gap of information about the importance of propagule pressure, residence time, and host community characteristics on these species natural establishment.

Understanding why some sites are more susceptible to the establishment of a given plant species than others has long been a central topic in biological invasions research. The establishment success of an exotic species depends on the characteristics of the new species itself and the host community, and on the interaction between them (Lonsdale, 1999; Rejmánek et al., 2005; Richardson and Pyšek, 2006), such that a species colonization will not succeed in all recipient areas. In turn, susceptibility of the receiving community can change in function of competitive and facilitative interactions, nutrient availability, or disturbance levels (e.g. Davis and Pelsor, 2001; Davis et al., 2000; Traveset and Richardson, 2014) (Fig. 1). Particularly, the role of disturbance have been long recognized as possible important driver of invasion (Elton, 1958). For instance, many studies have observed that disturbance, specifically native vegetation removal, favors exotic plants (e.g. Alston and Richardson, 2006; Davis and Pelsor, 2001; Davis et al., 2000; Higgins and Richardson, 1998; Mitchell et al., 2006), and has been suggested to be a prerequisite for eucalypts establishment (da Silva et al., 2011; Lorentz and Minogue, 2015; Wevill and Read, 2010).

The number of propagules arriving to the new environment (i.e. propagule pressure) has also been observed as one of the mechanism that strongly influences exotic species colonization success (e.g. Krivánek et al., 2006; Lockwood et al., 2009; Pyšek et al., 2009; Rouget and Richardson, 2003). In forestry species, the extension and the intensity of the planted area determine the magnitude of the propagule pressure that could affect the surrounding native vegetation (Fig. 1). *Eucalyptus* and *Pinus* propagule pressure can potentially be very high considering the extension of plantations and their prolific seed production (Rejmánek and Richardson, 2011). However, the importance of propagule pressure on establishment success can vary depending on the local conditions (i.e. characteristics of host community) (Fig. 1). For instance, a low amount of propagules can be sufficient to assure the colonization in a low resistant community (e.g. highly disturbed), whereas in locations with highly resistant communities (with intense competition or harsh abiotic conditions) it may be necessary a greater number of propagules (Krivánek et al., 2006; Lockwood et al., 2009; Pyšek et al., 2009; Rouget and Richardson, 2003). Similarly, residence time (i.e. the time since a species was first introduced into a region) can also influence the success of forestry species colonization (Dawson et al., 2009; Pyšek et al., 2009). Considering that data on these aspects is rarely available for most species introduced to a particular location, the existence of detailed records of planting history (and introduction) for forestry species offers an opportunity to access the relative importance of a potential surrogates for propagule pressure and residence time in explaining exotic species natural colonization success.

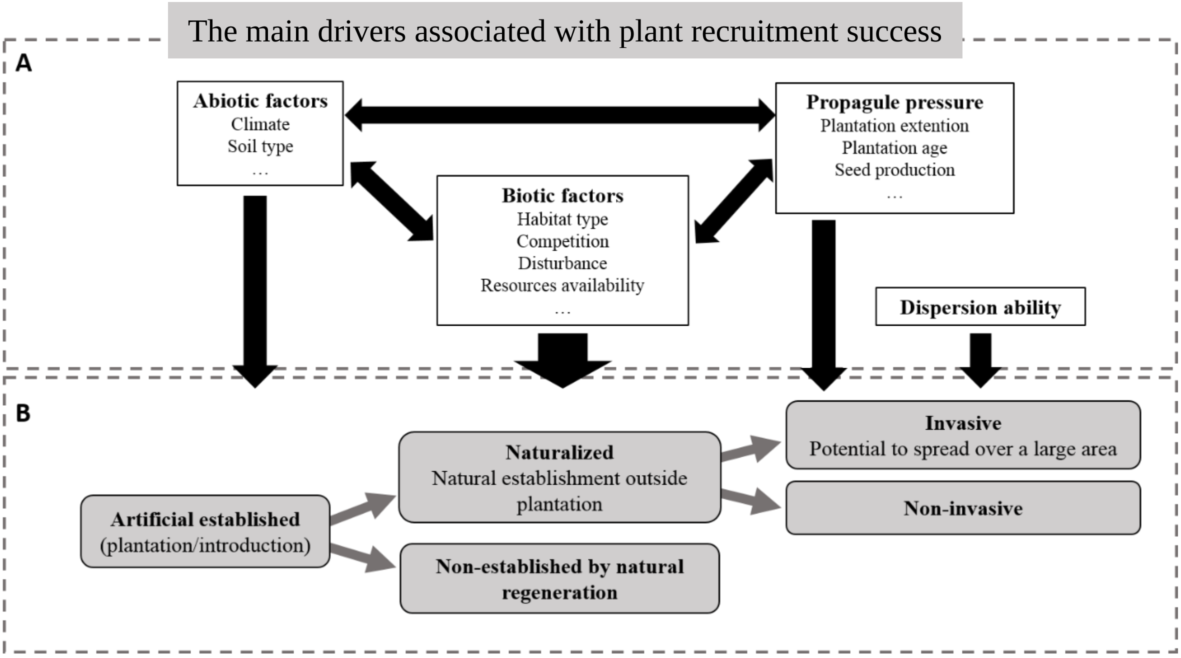


FIGURE 1. (A) Conceptual scheme summing up the main drivers to introduced plants establishment success and expansion, and (B) hierarchical scheme for the suggested classification of forestry species (see Box 1 for definition of terms). Abiotic factors are the main drivers to forestry plantation establishment. Forestry species are planted in accordance with their productivity under different edafo-climatic conditions. Their natural establishment succes (naturalization) is influenced by the combination of propagule pressure and a suitable environment, biotic and abiotic conditions for establishment. Finally their invasion potential will be determined by the capacity to produce large numbers of reproductive offspring (propagule pressure) at considerable distances from the parent tree (dispersion ability), and thus have potential to spread over a large area. Adapted from Catford et al., (2009), Chytrý et al. (2008a) and Pyšek et al., (2004).

The main forestry species in Portugal: Eucalyptus globulus and Pinus pinaster

In Portugal, planted forests for production of timber and paper products are predominantly composed of two species, *Eucalyptus globulus* Labill. and *Pinus pinaster* Aiton (ICNF, 2013). Both species are highly represented in Portugal mainland, which *E. globulus* represent 26% (812 thousand hectares) and *P. pinaster* 23% (714 thousand hectares) of its forest cover (ICNF, 2013). These species also have similarities on geographical range, mostly concentrated in the centre and north of the country (Fig.2). As an exotic cultivated species, *E. globulus* current distribution in country is the result of human activity.

Distribution of *Eucalyptus globulus* and *Pinus pinaster* in Portugal

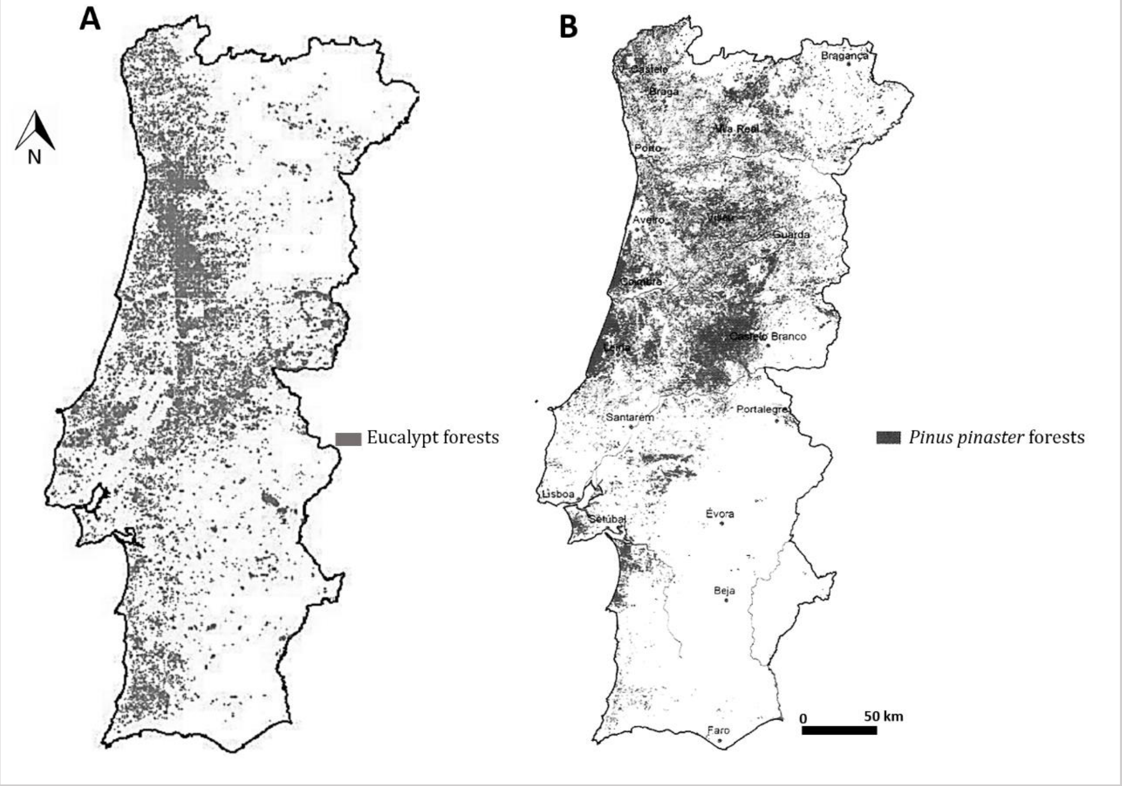


FIGURE 2. Map of *Eucalyptus globulus* main stands distribution in Continental Portugal (A); and map of *Pinus pinaster* main stands distribution in Continental Portugal (B). (Source: ICNF, 2013)

E. globulus, native from Australia, was introduced in the middle of the 19th century and since the middle of 20th century the development of the pulp and paper industries originated the expansion of *E. globulus* plantation in Portugal (Alves et al., 2007). Although it's planted area expansion came into being because of the pulp industry, this species had been introduced in Portugal in the late 19th century, either in 1854 or 1859, for timber production (Alves et al. 2007). The same authors also indicate that it had been used as an ornamental and medicinal species by private owners. Nowadays, *E. globulus* plantations are mostly intensive managed through a coppice system (10–12 year rotations). In turn, *P. pinaster* species is the most representative autochthonous species in Portugal, as confirmed by numerous archaeological and geological traces, for at least 33,000 years (Figueiral, 1995). The current pine distribution area had been strongly influenced by human activities and since the 19th century was used in large scale reforestation, due to its pioneer behavior in poor and rocky soils and it's late aptitude to the sawmills, panel board, resin, and pulp production (aims of the State forestry promotion plan in the first half of the 20th century), which lead to its expansion clearly beyond their natural distribution (Aguiar et al., 2007; Figueiral, 1995). In Figueiral *et al* (1995) is evident the difficulty in recognize the natural, historical and ecological distribution of *P. pinaster* in Portugal, mainly due to widespread plantation of the species which is not concurrent with its natural development. In the first forestry inventories (stating the names of the species and the area they covered) drawn during the 19th century is registered that, in 1879, *P. pinaster* occupied an area of only 5000 ha (Figueiral, 1995). Indeed, there is no doubt that today´s wide distribution results from forestry policy. Nowadays, commercial *P. pinaster* plantations are mainly used for wood production and it´s planted area registered a decrease in part due to pine wilt disease.

In Portugal, given their exotic origin and their widespread use in forestry, *E. globulus* has been recently a controversial species in relation to the presence or not of the invasive behavior. The invasion risk of *E. globulus* was assessed in several publications based on the Australian Weed Risk Assessment (Daehler et al., 2004; Gassó et al., 2009; Gordon et al., 2012; Marchante et al., 2014). However, weed risk assessments (WRA) are mainly based on species biological traits and expert knowledge (Pheloung et al., 1999), and rarely based on field quantitative information (distribution of the species in the introduced range). Thus, given the extend of *E. globulus* plantation (high propagule pressure), their fast growth, the production of very large quantities of seeds, and in light of their diverse adaptations for dealing with disturbance (such as fire), the invasion risk of this species was classified as “High” (based on the Australian WRA) in Spain (Gassó et al., 2009), in Portugal (Marchante et al., 2014), in the USA (Gordon et al., 2012) and, in Hawaii and other Pacific Islands (Daehler et al., 2004). Specifically, Marchante et al. (2014) field guide reported *E. globulus* as invader in Portugal without field quantitative information. In contrast with WRA classifications, field quantitative studies have reported *Eucalyptus* (including *E. globulus*) as a species with low invasive potential (Callaham et al., 2013; da Silva et al., 2011; Larcombe et al., 2013; Lorentz and Minogue, 2015). In general, *E. globulus* has very seldom spread considerable distances from planting sites, and their regeneration is frequently sporadic (Larcombe et al., 2013; Rejmánek and Richardson, 2011), and have high mortality of seedlings (Calviño-Cancela and Rubido-Bará, 2013; da Silva et al., 2011). These factors seem to be major reasons for the limited invasiveness of eucalypts (Rejmánek and Richardson, 2011). Understanding factors that may reducing or improving natural establishment in *E. globulus* should help in predicting the invasive potential of this species (Richardson and Rejmánek, 2011). In Portugal, the natural establishment of this species has been reported in the roadsides adjacent to plantations (Catry et al., 2015) and in burned plantations (Águas et al., 2014), although the values of eucalypts density in both studies is very low compared to well-known invasive forestry species (e.g. pines, Richardson et al., 1994). Recently, Catry et al. (2015) studied the effect of abiotic factors on *E. globulus* natural establishment along roadside transects adjacent to eucalypt plantations in continental Portugal. However, their study did not consider the characteristics of surveyed plantations and others important site variables, such as vegetation cover and disturbance level. Besides, roadsides adjacent to eucalypts plantations are areas of human-caused disturbance, becoming difficult to generalize to other habitat types. Therefore, we still have a gap of knowledge about the importance of propagule pressure, residence time, and the characteristics of the host community on *E. globulus* natural establishment. Additionally, the knowledge about their potential establishment from seeds and their expansion capacity into different habitats and ecological conditions is still almost inexistent.

In turn, *P. pinaster* is the main forest tree species for wood production in Iberian Peninsula (Garcia-Gonzalo et al., 2011a). Because of its commercial importance and easy acclimation, *P. pinaster* has been planted also in temperate regions outside its natural range. In fact, pine expansion is recognized as a global phenomenon (Richardson and Rejmánek, 2004). Outside its natural range, *P. pinaster* and several other pines are counted among the most invasive plant species, mainly in the southern hemisphere (Richardson and Rejmánek, 2011, 2004; Richardson, 1998). The spatial distribution of dispersed seeds of Mediterranean pine species, including *P. pinaster*, have been characterized as leptokurtic, with a peak very close to the source and declining sharply with distance, but with an extended tail of long distance dispersal (Higgins and Richardson, 1999; Rouget and Richardson, 2003). Still, given the importance of *P. pinaster* in south western Europe there are surprisingly few studies of its regeneration and expansion capacity in the Mediterranean (Juez et al., 2014; Rodríguez-García et al., 2011, 2010, 2007). The terms of invasion and invasive (Box 1) are mainly applied to exotic species, although native species can also reach the stages of spread of populations with impacts to ecology and economy (Catford et al., 2009). Indeed, *P. pinaster* can also show continuous recruitment with regeneration pulses over time in their native region (Rodríguez-García et al., 2011). Should, however, native pine expansion from plantations be seen as a part of the natural dynamics of the ecosystem? Or, although it occurs within its natural range, as a process caused by human activities with some negative consequences (i.e. invasion)? There is no consensus to this complex question (Valéry et al., 2009, 2008; Wilson et al., 2009). Alterations in ecosystem structure and function are one of the most reported effects of pine colonization into treeless areas, particularity in southern hemisphere (Higgins and Richardson, 1998). Indeed, the spread of pines outside plantation sites should also be a concern, whether encroaching from forests in the native range or from plantations in the introduced range (Taylor et al., 2016). In this sense, studies from pine natural range, especially those related to recruitment process can be very important for developing colonization models, increasing the insights from the native and introduced ranges on the distribution of pines (Richardson and Bond, 1991).

In both pines and eucalypts, the seeds are found within hard structures (Fig. 3; cones in the case of pines and capsules in eucalyptus) and dispersion of seeds are influenced by wind speed and fire (Juez et al., 2014; Reyes and Casal, 2001). However, the two species have very different seed dispersal abilities: *P. pinaster* seeds have large wings as a specialized wind dispersal structure and can be dispersed at long distances; *E. globulus* seeds have no adaptation for dispersal mechanism (wings or fleshy tissues) and are mainly dispersed within the capsules, and capsule dispersal by wind is presumed to be limited (Fig. 3; Calviño-Cancela and Rubido-Bará, 2013; Rejmánek and Richardson, 2011). In addition, *E. globulus* seeds are not known to be dispersed by animals. Both species are described as serotinous meaning that the fruits are able to withstand fire and be stimulated to release the seeds (Reyes and Casal, 2001). Both species seeds show non-dormancy, providing fast germination whenever conditions are favorable (i.e. high water availability and light) (Reyes and Casal, 2001, 1997). *P. pinaster* regular seed production initiates at 10-15 years age (in Iberian Peninsula) and large quantities of seeds are produced (Tapias et al., 2001). *E. globulus* sexual maturity occur earlier than *P. pinaster*, usually at 3-4 years old associated with the change to adult leaves (Jordan et al., 1999).

Although the ecology and physiology of these species is well known (Correia and Almeida, 2004; Correia et al., 1989; López et al., 2000; Porte and Loustau, 1998; Rodríguez-García et al., 2011; Sabaté et al., 2002; Schwanz and Polle, 2001), little is known about the regeneration and spread capacity of these species under different habitats and climatic conditions. Both species plantations are surrounded by different natural and semi-natural habitats, and their seeds are dispersed to a variety of microhabitats that provide a range of differences in biotic and abiotic conditions affecting seedling emergence, survival and growth, hence influencing establishment success (Castro et al., 2004; Niinemets and Valladares, 2006).

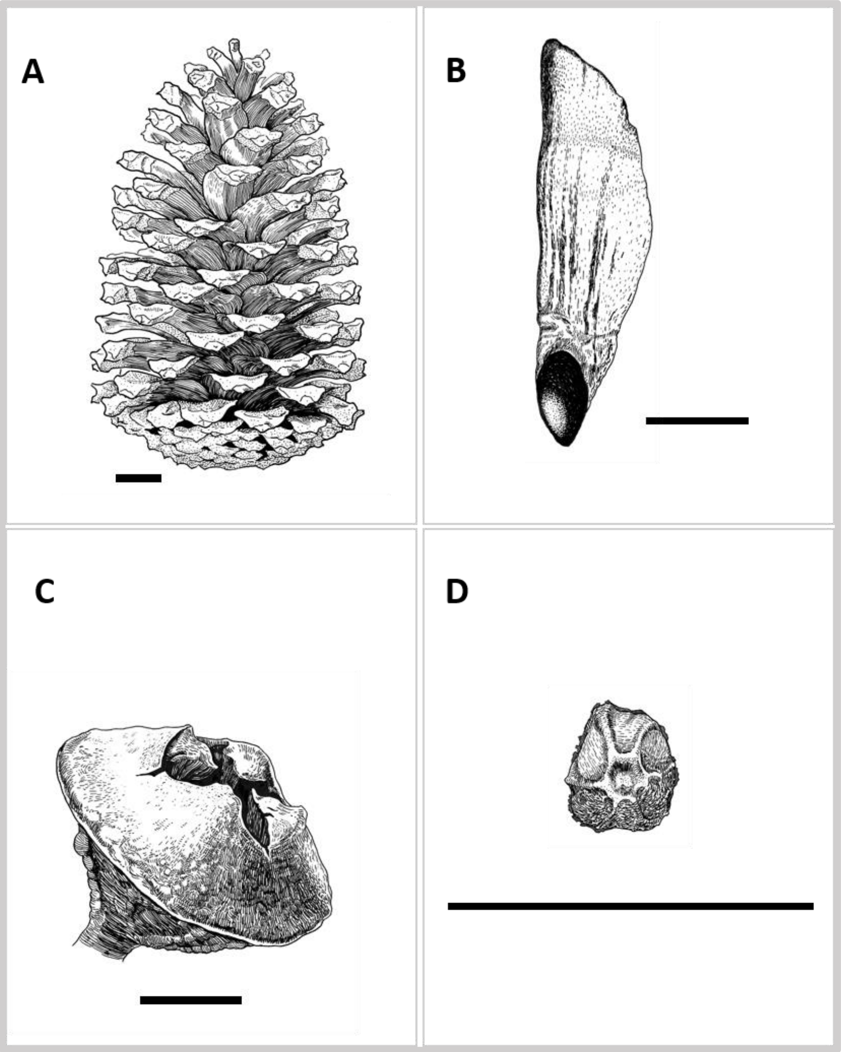


FIGURE 3. Ilustrations of *Pinus pinaster* cone (A) and seed with wing (B), and *Eucalyptus globulus* capsule (C) and seed (D) (illustrations were made by Catarina Costa, Francesco Milanese and José Perico). All size bar = 1cm.

GENERAL AIMS AND THESIS OUTLINE

The present thesis is focused in the evaluation of how variables related to a region´s climate, propagule pressure and host site characteristics (i.e. habitat type, soil type, aspect, disturbance level and vegetation cover) are associated with the natural establishment of the forestry species, *P. pinaster* and *E. globulus*, aimed to understand the natural regeneration and spread capacity of these species from plantations into natural habitats. In this thesis, climate was used to estimate abiotic suitability (Walther et al., 2009), plantation characteristics as a proxy to propagule pressure (Pyšek et al., 2009), and site characteristics (including habitat type, vegetation cover and disturbance level) mainly representing the characteristics of the host community (biotic and abiotic suitability) (Rejmanek et al., 2005).

The thesis includes four scientific papers published or submitted in peer reviewed international journals, each corresponding to a chapter. The specific objectives and main methodological approaches of each chapter are described below:

Chapter 2. **Natural regeneration of *Pinus pinaster* and *Eucalyptus globulus* from plantation into adjacent natural habitats.** The main objective of this chapter was to identify the recruitment spatial patterns of *P. pinaster* and *E. globulus* into habitats surrounding the plantations, and to determine the main factors influencing that recruitment pattern. More specifically we asked: (i) how is the seedling recruitment in relation to distance from the parent (plantation)?; (ii) which are the most important factors related with plantation characteristics, habitat type and climatic conditions influencing their recruitment spatial pattern and (iii) how do these variables affect each species recruitment spatial pattern? This was done by looking at recruitment success in several habitats near plantations, using a gradient of distance to plantation's edge. In total, 256 transects were performed along the 15 *Pinus pinaster* plantations, and 749 transects along the 23 *E. globulus* plantations, in the Center and North of Portugal.

Chapter 3. **What drives *Eucalyptus globulus* natural establishment outside plantations? The relative importance of climate, propagule pressure, and site characteristics.** The main objective of this chapter was to determine the main drivers of *E. globulus* establishment (occurrence and density). The specific questions were: i) what is the relative importance of site characteristics, propagule pressure, and climate in the natural establishment of eucalyptus from plantations? ii) once eucalyptus is established, which are the factors associated with the degree of colonization in natural habitats? For that, a specific model selection method was applied: Multimodel inference (Burnham and Anderson, 2002). This method provides a framework to 'evaluate the relative strength of evidence in data for multiple hypotheses represented as models' (Hobbs and Hilborn, 2006). Thus, multimodel inference method allowed to identify the best possible models and rank all explanatory variables according to their importance on eucalyptus occurrence and density (Burnham and Anderson, 2002). In total, 50 *E. globulus* plantations (1579 plots) were sampled in a country-wide field survey to document seedling establishment (natural regeneration) in the surrounding areas of the managed forest plantations in Portugal.

In both Chapters 2 and 3, the sampled plantations were surrounded by natural habitats (at least one edge in contact with natural habitat) and were in a reproductive age. The surrounding natural habitats were natural Forest (mainly small woods with *Quercus robur*, *Q. pyrenaica*, *Q. faginea*, *Q. suber,* and *Q. ilex*); mediterranean shrubland composed by a mix of short and medium shrubs (until 2 m of height) as rockrose (*Cistus spp.*) and others Cistaceae, heather (genus *Erica* and *Calluna*), gorse (*Ulex* spp), broom (*Cytisus* spp) and many aromatic species (e.g. *Lavandula stoechas*, *Rosmarinus officinalis*, *Thymus vulgaris*); and grassland with high diversity annual herbaceous species. Shrublands were the dominant habitat type surrounding forest plantations, as a result of agricultural land abandonment. In both chapters, the selected method for conducting a detection survey was to traverse the natural vegetation following a series of parallel transect established perpendicular to the plantations boundary edge, recording *P. pinaster* or *E. globulus* seedlings established by natural regeneration. This method was chosen because it allows a high level of detection, being designed to be effective for the detection of rare events (Green and Young, 1993).

Chapter 4. **Combined effects of climate, habitat and disturbance on seedling recruitment and survival of *Pinus pinaster* and *Eucalyptus globulus*.** The aim of this chapter was to evaluate the establishment capacity of *P. pinaster* and *E. globulus* in an array of ecological conditions, and identify those conditions that favor or hamper these species establishment (that might contribute to their spread capacity). The establishment success was measured in terms of seedling emergence and seedling survival capacity as these traits have been found to be related with invasive capacity (Calviño-Cancela and Rubido-Bará, 2013; González-Muñoz et al., 2011; Niinemets and Valladares, 2006). The effects of disturbance (vegetation removal), habitat type (forest and shrubland) and climate (mesic and xeric conditions) on seedling emergence and survival were investigated using a seed addition experiment. The specific question was: how do the combinations of these factors affect the establishment success of these species? This information was crucial to better understand the results found in the two chapters described above.

Chapter 5. **Natural regeneration in unmanaged eucalypt and pine plantations in Portugal: potential of plantations in harboring natural vegetation**. The main objective of this chapter was to measure the recruitment structure of these two planted species and, the understory species richness and cover across three different unmanaged forest types: (i) *E. globulus* pure plantations; mixed *E. globulus* and *P. pinaster* plantation with eucalypt dominant; and mixed *E. globulus* and *P. pinaster* plantation with pine dominant. Understory plants are used here as appropriated biodiversity indicators as their diversity is often correlated with that of other taxonomic groups (Rodrigues and Brooks, 2007). Thus, in this chapter it were assessed by one hand the value of these forest types to harbor natural vegetation and secondly the potential regeneration of these two planted species, *E. globulus* and *P. pinaster*. In both chapters 2 and 3, the surveys were performed in the managed plantations. However, in the northern and centre of Portugal we also have many mixed *E. globulus* and *P. pinaster* forests with very low professionalization and rudimentary management, which is considered far from optimal in terms of productivity. Nevertheless, it brings the opportunity to study the occurrence of understory plant species and structural patterns under non-existent management, thus allowing to better isolating the effects of the plantation itself from those caused by management practices.

Finally, **chapter 6** outlines the main conclusions gathered from the chapters described above, their contributions and implications within the context of the natural regeneration and expansion of these forestry species into natural habitats. This chapter also point out some recommendations on how management practices can be optimized in order to avoid an undesirable colonization by these species.

This thesis was performed in partnership between Faculdade de Ciências da Universidade de Lisboa and The Navigator Company. The Navigator Company offered logistical and data support which allowed to access detailed information about plantations and to produce a comprehensive data base.

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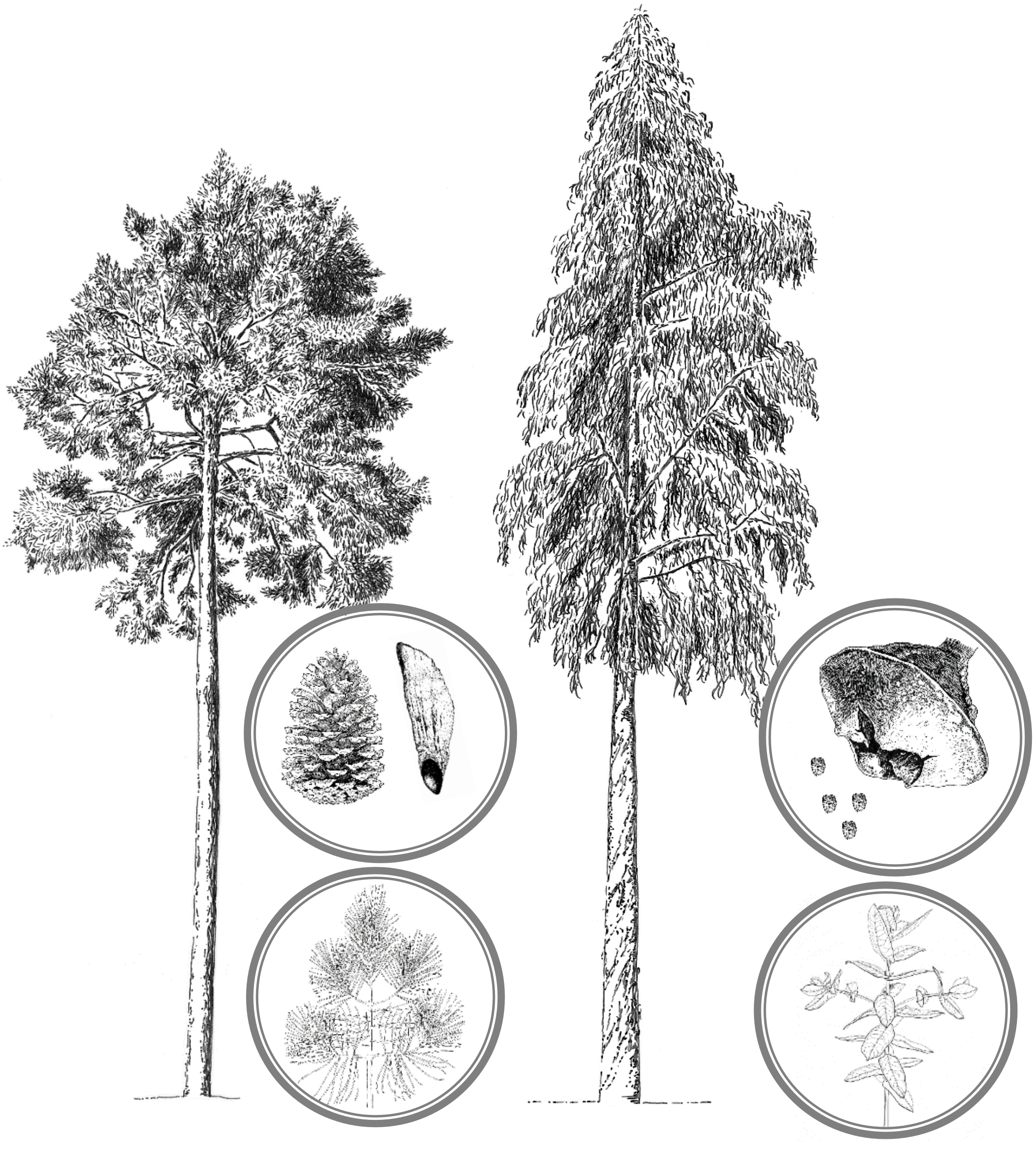
CHAPTER 2

NATURAL REGENERATION OF *PINUS PINASTER* AND *EUCALYPTUS GLOBULUS* FROM PLANTATION INTO ADJACENT NATURAL HABITATS



Fernandes, P., Antunes, C., Pinho, P., Máguas, C. & Correia, O. (2016). Natural regeneration of *Pinus pinaster* and *Eucalyptus globulus* from plantation into adjacent natural habitats. *Forest Ecology and Management* 379, 91-102. doi: 10.1016/j.foreco.2016.07.027

ABSTRACT

The tree species used in planted forests are highly productive and have an important role in countries economy but the expansion of these trees into habitats outside plantations is a concern for managers and conservationists. Among the most planted forest species worldwide, we can find eucalypts and pines species. Understanding spatial patterns and the factors that influence these species colonization is crucial to increase the knowledge about expansion capability of these species and may help managers to improve and prioritize eventual control plans. Our study aimed to identify the recruitment spatial patterns of *Pinus pinaster* (native species) and *Eucalyptus globulus* (exotic species) into habitats surrounding plantations in Portugal (native forests, grasslands and shrublands) and to determine the factors that influence recruitment. This was done by looking at the recruitment success in several habitats near plantations, using a spatial gradient of distance to plantations edge. We observed as expected that the recruitment of *P. pinaster* and *E. globulus* decreased exponentially from the plantation edge. However, the higher *P. pinaster* seedling recruitment in this study (*P. pinaster*: 247 seedlings/ha; *E. globulus*: 22 seedlings/ha), the smallest decline in seedlings density with distance from plantation boundary (*P. pinaster* = -0.036 seedlings/m vs *E. globulus* = -0.048 seedlings/m), and the longer distance of seedling establishment, demonstrate clearly that *P. pinaster* is more successful colonizing the habitats near plantations than *E. globulus*. Nevertheless *E. globulus* can growth seedlings within the planted stands suggesting that the species is becoming naturalized. However, localized recruitment (92% of the total seedlings recorded were located less than 15m from the plantation edge) and lower levels of establishment of *E. globulus* from managed plantations, suggested that this species did not demonstrate an invasive behavior. However, future research in abandoned eucalyptus plantation is needed to understand the role of plantation management and age as barriers to *E. globulus* colonization. Results from our survey revealed the suitability of all habitat types studied for *P. pinaster* natural regeneration, although their recruitment was more limited in forest habitat type. Forest and grassland were very resistant to *E. globulus* establishment. The two species recruitment was also influenced by different factors, with *P. pinaster* being independent of climatic variables, while *E. globulus* was affected by temperature seasonality and recruitment was found to be higher in areas with lower seasonal differences.

INTRODUCTION

During the last decades the increasing world demand for forest products (such as timber, fuel and paper) and the needs to restore large degraded areas has led to a global increase in planted forest area (Dodet and Collet, 2012; FAO, 2010). In many countries, plantation forestry consist mostly on a few fast-growing species, mainly eucalyptus and pines (Richardson, 1998). Once planted, these fast-growing species can spread to nearby natural and semi-natural ecosystems by seed regeneration.

Regeneration from seeds involves many stages in the life cycle of plants, with seed and seedling stages recognized as the most important for their success (Houle, 1996). However, the success of regeneration and establishment of a new species is context specific and depends on both characteristics of the new species itself and of the host community and on the interaction between the two (Rejmánek et al., 2005). Thus, the ability of introduced species to recruit, the capacity of a species to reach suitable regeneration sites (which depends on the number of seeds produced and on the dispersal ability), and the susceptibility of the environment (or its opposite, ecological resistance) to the establishment of these new individuals are crucial for the recruitment success (Lonsdale, 1999). Davis et al. (2000) suggests that the susceptibility of a receiving community can change in function of competitive and facilitative interactions, nutrient availability, or disturbance and can be quantified as the probability of establishment per arriving propagule of the new individual. Thus, understanding the spatial pattern of establishment of new individuals (recruitment) of introduced forestry species is an important prerequisite to manage their colonization into natural habitats.

Among the most planted forest species worldwide, we can find Eucalyptus and Pinus species. In northern and central of continental Portugal, most forest area is due to the plantation of two species, *Eucalyptus globulus* Labill. (Tasmanian blue gum) and *Pinus pinaster* Aiton (maritime pine) (ICNF, 2013).

P. pinaster is a species from western Mediterranean Basin that covers over four million hectares and has great economic and ecological importance in this region (Ribeiro et al., 2001). *P. pinaster* was used in Portugal since the 19th century in large scale reforestation, which lead to its expansion clearly beyond their natural distribution (Aguiar et al., 2007). Actually, this species is the main forest tree species for timber production in Portugal

(Garcia-Gonzalo et al., 2011a), representing 23% of its forest cover (ICNF, 2013). Still, given the importance of *P. pinaster* in south western Europe there are surprisingly few studies of its regeneration in the Mediterranean (Juez et al., 2014; Rodríguez-García et al., 2011, 2010, 2007). Because of its commercial importance and easy acclimation, maritime pine has been planted also in temperate regions outside its natural range. In fact, pine expansion is recognized as a global phenomenon (Richardson and Rejmánek, 2004). Outside its natural range, maritime pine and several other pines are counted among the most invasive plant species, mainly in the southern hemisphere (Richardson and Rejmánek, 2011, 2004; Richardson, 1998). Should, however, native pine expansion from plantations be seen as a part of the natural dynamics of the ecosystem? Or, although it occurs within its natural range, as a process caused by human activities with some negative consequences (i.e. invasion)? There is no consensus to this complex question (Valéry et al., 2009, 2008; J. R. U. Wilson et al., 2009). In this sense, studies from pine natural range, especially those related to recruitment process can be very important for developing colonization models (increasing the insights from the native and introduced ranges on the distribution of pines) (Richardson and Bond, 1991).

E. globulus, native from Australia, is the most important pulpwood species planted in the Iberian Peninsula (Potts et al., 2004). This species has a significant economic role in this region (Potts et al., 2004) and is now the most widespread tree species in Portuguese mainland, representing 26% of its forest cover (ICNF, 2013). This species was introduced in the middle of the 19th century and since the middle of 20th century the development of the pulp and paper industries originated the expansion of *E. globulus* plantation in the country (Alves et al., 2007). But, as other widely planted species such as acacias and pines, there are concerns about the *E. globulus* natural regeneration from seeds. However, quantification of tree establishment has rarely been implemented for eucalyptus (Callaham et al., 2013; da Silva et al., 2011), and very few deal with *E. globulus* (Calviño-Cancela and Rubido-Bará, 2013; Catry et al., 2015; Larcombe et al., 2013).

The invasion risk of *E. globulus* was assessed in several publications based on the Australian Weed Risk Assessment (Daehler et al., 2004; Gassó et al., 2009; Gordon et al., 2012; Marchante et al., 2014). Weed risk assessments (WRA) are mainly based on species biological traits and expert knowledge (Pheloung et al., 1999), and rarely based on field quantitative information (distribution of the species in the introduced range). Given the extend of *E. globulus* plantation (high propagule pressure), their fast growth, the production of very large quantities of seeds, and in light of their diverse adaptations for dealing with disturbance (such as fire), the invasion risk of this species was classified as "High" (based on the Australian WRA) in Spain (Gassó et al., 2009), in Portugal (Marchante et al., 2014), in the USA (Gordon et al., 2012), in Hawaii and other Pacific Islands (Daehler et al., 2004). Indeed, Rejmánek and Richardson (2013) showed that *E. globulus* was reported as invasive in seven of the fifty geographical regions considered by these authors: North, Central and South America, Europe, New Zealand, Pacific Islands and Indian Oceans Islands. Namely, *E. globulus* was reported as invasive in Europe based on Marchante et al. (2014) field guide (where *E. globulus* is classified as a major invader in Portugal based on Australian WRA) (Rejmánek and Richardson, 2013).

On the other hand, *Eucalyptus* have been reported as species with a low invasive risk based on field quantitative studies (Callaham et al., 2013; da Silva et al., 2011; Larcombe et al., 2013; Lorentz and Minogue, 2015). Recently, Catry et al. (2015) studied *E. globulus* natural establishment along roadside transects adjacent to eucalypt plantations distributed throughout continental Portugal. They found that *E. globulus* density was mainly affected by precipitation, reaching the highest values at around 1500 mm of annual precipitation and the lowest values below 800 mm and above 2400 mm (Catry et al., 2015). In addition, *E. globulus* establishment was also more abundant in areas with lower thermal amplitudes and seemed to be favoured in areas with certain soil types (Cambisols and Podzols) (Catry et al., 2015). However, roadsides adjacent to eucalypt plantations are areas with particular characteristics, such as high human-caused disturbance, becoming difficult to generalize to other habitat types. We still have an information gap about recruitment spatial pattern and the factors that influence this species colonization from plantations into different natural habitats.

Whereas a lot is known of the invasion ecology of pines (e.g. summarized in Richardson, 2006), very little is known about crucial aspects (such as seed dispersal, and the role of fire and mycorrhiza) that determine the limited range of eucalypts natural regeneration (Rejmánek and Richardson, 2011). In the light of this, and in order to provide guidelines for better managing plantations and its surrounding natural and semi-natural habitats, the main goal of our study was to identify the recruitment spatial patterns of *P. pinaster* and *E. globulus* in to habitats surrounding the plantations, and to determine the factors influencing that recruitment. More specifically we asked: (i) how is the seedling recruitment in relation to distance from the parent (plantation)?; (ii) which are the most important factors related with plantation characteristics, habitat type and climatic conditions influencing their recruitment spatial pattern and (iii) how do these variables affect each species recruitment spatial pattern? This was done by looking at recruitment success in several habitats near plantations, using a gradient of distance to plantation's edge.

METHODS

Study species

Despite *Pinus pinaster* being a Portuguese native species it was included in this study because, as *Eucalyptus globulus*, this species is highly represented in Portugal mainland and, as a cultivated tree, its current distribution in Portugal is mainly a result of human activity. In Portugal, *E. globulus* plantations are mostly intensive managed through a coppice system (10–12 year rotations) and their wood is mainly used for pulp production (Soares et al., 2007). The main limiting climatic factors to *E. globulus* growth in Portugal are water availability and low temperatures (mainly occurrence of temperatures below 0ºC) (Almeida et al., 1994; Alves et al., 2012). In *P. pinaster* forests, final cut occurs around 45 years after plantation establishment and is mainly used for wood production. In both pines and eucalyptus, the seeds are found within hard structures (cones in the case of pines and capsules in eucalyptus) and dispersion of seeds are influenced by wind speed and fire (Juez et al., 2014; Reyes and Casal, 2001). Both species are described as serotinous meaning that the fruits are able to withstand fire and be stimulated to release the seeds (Reyes and Casal, 2001). *P. pinaster* seeds have large wings as a specialized wind dispersal structure and can be dispersed to long distances. In opposition, *E. globulus* seeds are smaller and with no specialized dispersal mechanism. Cremer (1977) based on *E. globulus* seed weights concluded that this species has a limited dispersal capacity when compared with others eucalyptus species, mostly limited to a radius of c. twice the tree height. Moreover, *E. globulus* seeds are mainly dispersed within the fruits, capsules are the main diaspores (Calviño-Cancela and Rubido-Bará, 2013). *P. pinaster* regular seed production initiates at 10-15 years age (in Iberian Peninsula) and large quantities of seeds are produced (Tapias et al., 2001). *E. globulus* sexual maturity occur earlier than *P. pinaster*, usually at 3-4 years old associated with the change to adult leaves (Jordan et al., 1999) and each capsule contains c. 6-25 viable seeds (Hardner and Potts, 1995; Mimura et al., 2009). The viability of seeds its very irregular and depends on the environmental conditions where they are found (Burrows and Burrows, 1992; López et al., 2000).

Study sites

We undertook field surveys to document seedling establishment (natural regeneration) in the surrounding areas of the managed forest plantations of *Pinus pinaster* and *Eucalyptus globulus* across Centre and North of Portugal (Fig.1A). In total, we sampled 15 *P. pinaster* plantations, ranging from 0.9 ha to 10.7 ha (average site area = 4.1 ha), and 23 *E. globulus* plantations, ranging from 0.5 to 15.7 ha (average site area = 4.1 ha) (Table A1 and A2 in appendix). The average elevation is 466 m a.s.l (ranging from 257 to 659 m) and 390 m a.s.l (ranging from 127 to 706 m) in the *P. pinaster* plantations and *E. globulus* plantations, respectively (Table A1 and A2 in appendix).

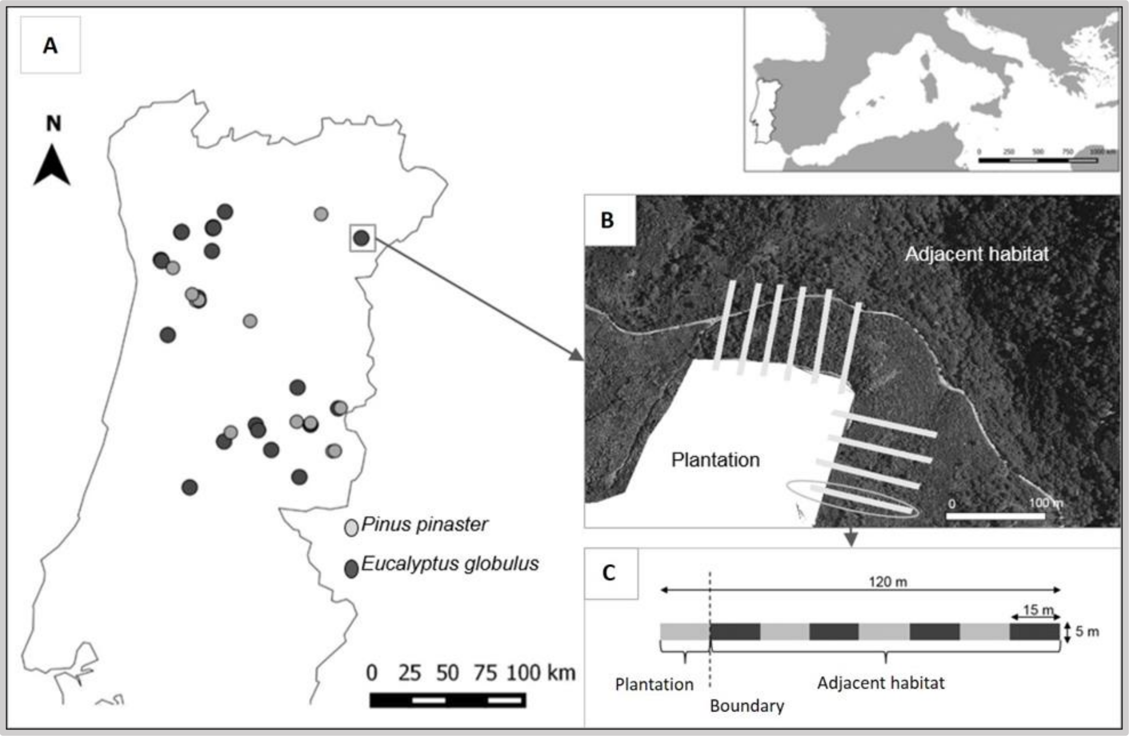


FIGURE 1. Location of the 15 studied *Pinus pinaster* plantations (in grey dots) and 23 studied *Eucalyptus globulus* plantations (in dark grey dots) in Portugal (**A**). **B**) Grey lines perpendicular to the plantations boundary edge correspond to survey transects. **C**) Schematic diagram of transects design. The beginning of each belt transect was established from 15 m inside the stand boundary, extending into the adjacent vegetation until the maximum length of 120 m. Each transects consisted of 8 distance plots of 15m x 5 m (75 m^2): 1) inside the stand boundary; 2) 0 to 15 m; 3) 15 to 30 m; 4) 30 to 45 m; 5) 45 to 60 m; 6) 60 to 75 m; 7) 75 to 90 m and 8) 90 to 105 m from stand boundary.

We selected sites with moderate topography, i.e., slopes ranging from 0 to 10%, in order to minimize the effect of slope in the relation of seedling density among sites and between species. The soil types found among sampled sites are cambisol (10 *P. pinaster* plantations and 14 *E. globulus* plantation) and lithosol (5 *P. pinaster* plantations and 9 *E. globulus* plantation) (Table A1 and A2 in appendix). The climate is Mediterranean: cool rainy winters and hot dry summers. The average of annual mean temperature of the selected sites is 14ºC (in the both *P. pinaster* and *E. globulus* sites). The mean temperature of warmest months (July, August and September) is 21ºC (in the both *P. pinaster* and *E. globulus* sites) and the mean temperature of coldest months (December, January and February) is 7ºC in *P. pinaster* sites and 8ºC in *E.globulus* sites (Table A1 and A2 in appendix). The annual precipitation is in average 1027 mm in *P. pinaster* sites (ranging from 748 mm to 1370 mm) and 1103 mm in *E. globulus* sites (ranging from 636 mm to 1438 mm) (Table A.1 and A.2 in appendix – Supplementary material).

We targeted plantations adjacent to natural habitats (at least one edge in contact with natural habitat) and of a reproductive age (this information was obtained from the plantation owners) and both burnt and unburnt plantations (Table A.1 and A.2 in appendix Supplementary material). The surrounding natural habitats were natural Forest (mainly small woods with *Quercus robur, Q. pyrenaica* and *Q. faginea*); shrubland (mediterranean garrigue) composed by a mix of short and medium shrubs (until 2 m of height) as rockrose (*Cistus spp.*) and others Cistaceae, heather (genus *Erica* and *Calluna*), gorse (*Ulex* spp), broom (*Cytisus* spp) and many aromatic species (e.g. *Lavandula stoechas, Rosmarinus officinalis, Thymus vulgaris*); and grassland with high diversity annual herbaceous species. Shrublands were the dominant habitat type surrounding forest plantations, as a result of agricultural land abandonment. The average age of selected *P. pinaster* plantations was 26 years, ranging from 20 to 36 years, and the *E. globulus* plantations have an average age of 15 years, ranging from 10 to 26 years (Table A1 and A2 in appendix). The burnt plantations were mainly a result of the big fires events of 2003 and 2005.

Field sampling

The field surveys were conducted between May 2012 and May 2014, during spring and autumn. The selected method for conducting a detection survey was to traverse the natural vegetation following a series of parallel transect established perpendicular to the plantations boundary edge, recording *P. pinaster* or *E. globulus* seedlings established by natural regeneration (Fig. 1B). Based on Callaham et al. (2013), the area being surveyed was a belt transect with 5 m width and 120 m length (600 m^2). The beginning of each belt transect was established from 15 m inside the stand boundary, extending into the adjacent vegetation until the maximum length of 120 m (Fig. 1C). Each transects consisted of 8 distance plots of 15m x 5 m (75 m^2) (Fig. 1C): 1) inside the stand boundary; 2) 0 to 15 m; 3) 15 to 30 m; 4) 30 to 45 m; 5) 45 to 60 m; 6) 60 to 75 m; 7) 75 to 90 m and 8) 90 to 105 m from stand boundary (Fig. 1C). This method was chosen because it allows a high level of detection, being designed to be effective for the detection of rare events (Green and Young, 1993), and the seedlings occurrence is registered along a distance transect from the plantation edge. Other studies used similar approaches to detect tree wildlings in the surrounding areas of the plantations (Callaham et al., 2013; Lavi et al., 2005). To calculate the confidence level of the survey, i.e. the probability of sampling seedling establishment at least once given a particular value of transects, we applied the following equation (for more detailed information about the equation see Callaham et al. 2013 and Green and Young, 1993) that gives estimates of how many samples (in this case, transects) must be taken to achieve detection of events (in this case, seedling establishment) (Green and Young, 1993):

$$n = -\frac{1}{m} * (\log \beta),$$

were, n = the number of transects needed to detect seedling establishment, *m* = the mean frequency of occurrence of seedling establishment, and β = confidence level of the estimate. In practice, it´s very difficult to determine *m* before the end of the sampling, and therefore must be operationally defined a priori. So we adopted *m* = 0.1 for both species as one definition of “rare” suggested by Green and Young (1993). Based on this equation we calculated the number of transects needed to achieve a high confidence level (the value of β). Before the survey beginning, we determine a realistic number of 30 transects per site resulting in a confidence level equal to 0.95. The spacing of transects at each stand were determined by taking the total length of the stand boundary and dividing by 30, with the resulting value used as a standard distance between sampling transects. In the case of very small stands (when the stand perimeter was less than 500m), the distance between transects was set to approximately 15m. In some cases, and mainly due to boulders, ravines, steep slopes and very dense vegetation, was not possible to perform transects and traverse the natural vegetation and those transects were eliminated from the analysis. In addition, transects were only performed when the target plantation was the only nearby seed source to the observed habitat, in order to ensure that any seedling found inside the transect could not have originated from another source (i.e. a second plantation or paddock tree). In this sense, the number of transects at each site varied in function upon the size of the sampled plantation, the accessibility of adjacent habitat and the distance to others seedlings sources.

The total number of transects ranged from 4 to 33 transects per *P. pinaster* plantation and from 11 to 51 transects per *E. globulus* plantation (Table A3 in appendix). Consequently the values of β (the calculated probability of actually sampling a seedling given the number of transects sampled, and an assumed frequency of 0.1) varied from site to site. The values of β for our survey is in average 0.76 for *P. pinaster* sites and 0.94 for *E. globulus* sites and are reported for individual sites in Table A3 in appendix.

All established seedlings were registered in each plot and categorized to one of two size classes based on their height: 0-1 m (classified as young seedlings) and 1-3 m (classified as old seedlings). Seedlings density was calculated for each distance plot along transects. Normally, the plantation boundary is immediately surrounded by a dirt track without any vegetation with a width from 3m to 5m. So, for the calculation of seedlings density inside the first distance plot outside the plantation (0-15m) we didn´t count the area with dirt track cover.

Data analysis

Mean seedling density was plotted against distance from the plantation edge (distance from the edge to the centre of each distance plot) to obtain 'recruitment curves' (i.e. spatial pattern of seedling establishment) for each species and habitat. Seedling density was estimated by averaging the densities from each distance plots. The negative exponential model (ln [density + 1] versus distance) were fitted to these curves (Lavi et al. 2005; Nathan et al. 1999). Models were fitted by least-squares regression after suitable transformation of variables.

The same method was applied to each habitat type by plantation, fitting a negative exponential equation ($\log(y) = a + b\,(x)$) to the observed 'recruitment curves' in pines and eucalypts in each sampled site. Only plantations with plant establishment were considered. We obtain 21 *P. pinaster* 'recruitment curves' (one for each habitat by plantation). *E. globulus* ‘recruitment curves’only included the shrubland habitat since the number of transects with presence of eucalyptus seedlings, as well as the density (Fig. 4), were too small in the forest and grasslands to be modelled. The final data set of *E. globulus* included 16 ‘recruitment curves’ (one for each plantation with seedlings presence in shrubland transects).

In order to identify factors that influence the ‘recruitment curves’ across the plantations survey, three response variables were modelled using generalized linear models (GLMs): the regression slope of the exponential model (regression slope model), the intercept b (ln density at plantation boundary – density model) and maximum distance from plantation boundary with seedlings presence (distance model). Bioclimatic variables, soil type, habitat type (only for *P. pinaster* models) and plantation characteristics (plantation size, age and fire occurrence) were used as explanatory variables.

Average monthly temperature, maximum and minimum temperature, average monthly precipitation, average altitude and 19 other bioclimatic variables were extracted from the WORLDCLIM interpolated map database with a 30 arc-seconds (~1km) resolution (Hijmans et al., 2005). Prior to modelling, we checked the collinearity among bioclimatic variables by pair-wise Pearson’s correlation tests. First, we selected variables that had a pair wise correlation lower than 0.6 and then selected ones with best explanatory power (González-Moreno et al., 2013a). The result was a final set of tree bioclimatic variables for each species: minimum temperature of coldest quarter (ºC), precipitation seasonality and annual precipitation (mm) for *P. pinaster* models (Table A.4 in appendix - Supplementary material); temperature seasonality, mean temperature of coldest quarter (ºC) and annual precipitation (mm) for *E. globulus* models (Table A.5 in appendix - Supplementary material).

For both species, the response variables were modelled using a gaussian distribution and an identity link. Model selection was based on a backward step model simplification process (Zuur et al., 2009), starting with a model that included all explanatory variables and sequentially removing the least significant variables (with the largest *P value*) to the explained deviance.

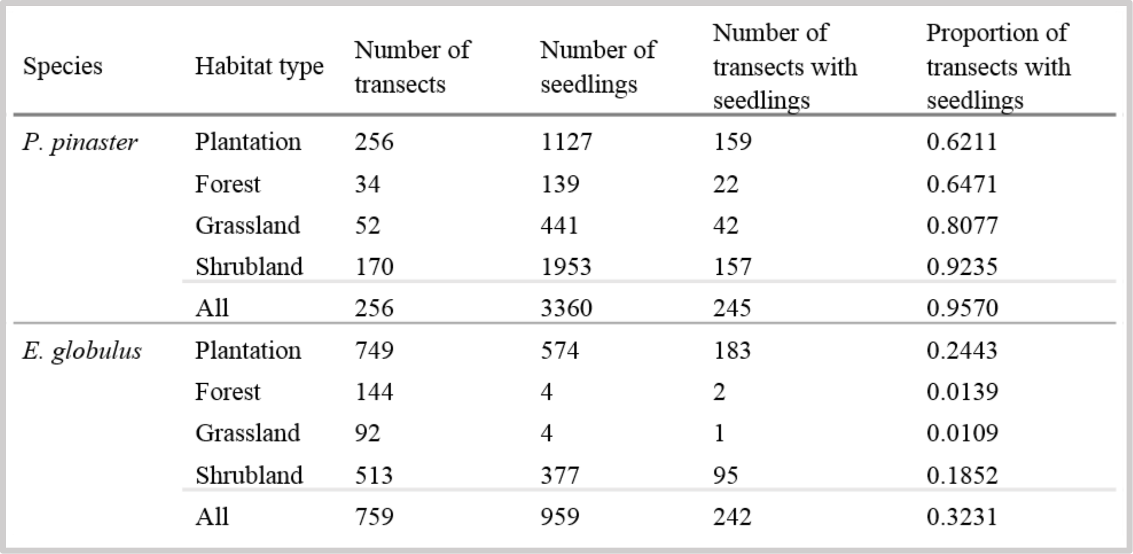
All analyses were performed on R statistical software V.3.1.1 (R Development Core Team, 2014) using the R Studio V 0.97.312 interface.

RESULTS

Quantification of seedlings in relation to distance gradient

In total, 256 transects (14.8 ha of surveyed area) were performed along the 15 *Pinus pinaster* planted sites and 749 transects (43.9 ha of surveyed area) along the 23 *E. globulus* planted sites, in the Center and North of Portugal (Table A.3 in appendix). The survey resulted in a detection of 3660 seedlings of *P. pinaster* and 959 seedlings of *E. globulus* (Table 1; Table A3 in appendix), giving a study wide average of 247 *P. pinaster* seedlings per ha and 22 *E. globulus* seedlings per ha. This means that, in total, the seedlings density of *P. pinaster* was eleven times higher than the density registered for *E. globulus*.

TABLE 1. A summary of the habitat types at each sampled transect from all sites surveyed, and the number of seedlings present among the habitat types. The habitat type Plantation - represents the plots sampled inside the plantation of interest and it´s the beginning of all performed transects. Forest, grassland and shrubland represents the habitats adjacent to sampled plantations.



Species	Habitat type	Number of transects	Number of seedlings	Number of transects with seedlings	Proportion of transects with seedlings
P. pinaster	Plantation	256	1127	159	0.6211
	Forest	34	139	22	0.6471
	Grassland	52	441	42	0.8077
	Shrubland	170	1953	157	0.9235
	All	256	3360	245	0.9570
E. globulus	Plantation	749	574	183	0.2443
	Forest	144	4	2	0.0139
	Grassland	92	4	1	0.0109
	Shrubland	513	377	95	0.1852
	All	759	959	242	0.3231

P. pinaster seedlings presence was recorded on 245 transects, representing 96% of the total transects surveyed performed (Table 1). *E. globulus* seedlings presence was recorded on 242 transects, representing 32% of the total transects (Table 1). Seedlings density per distance plot showed differences in seedlings distribution along the transect length between species (Fig. 2). *P. pinaster* seedlings were mostly observed outside of the stands as far as 105m away (69% of the total seedlings were registered after the plantation boundary).

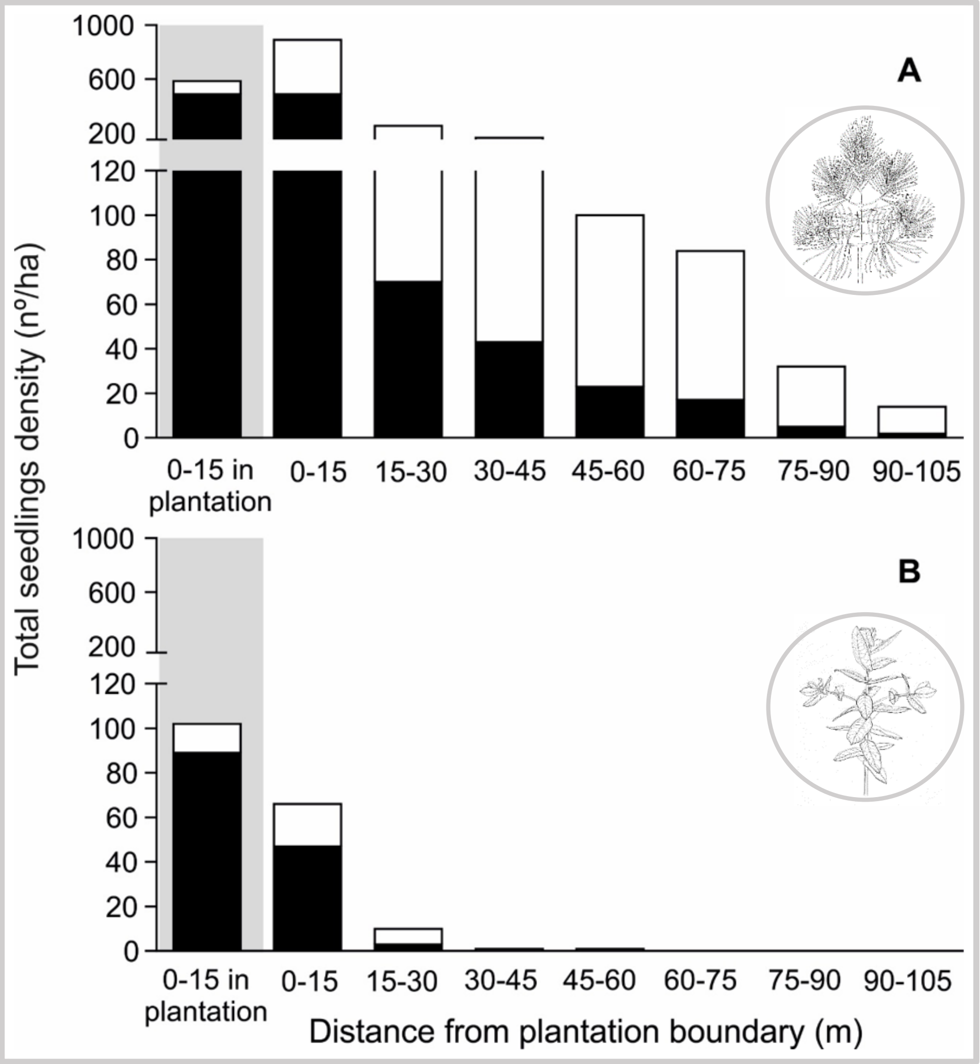


FIGURE 2. Total seedlings density (seedlings/ha) distribution in relation to distance from plantation boundary (m). **A)** *Pinus pinaster*; **B)** *Eucalyptus globulus*. The white part of the bars represents the density of old seedlings and the black part represents the density of young seedlings.

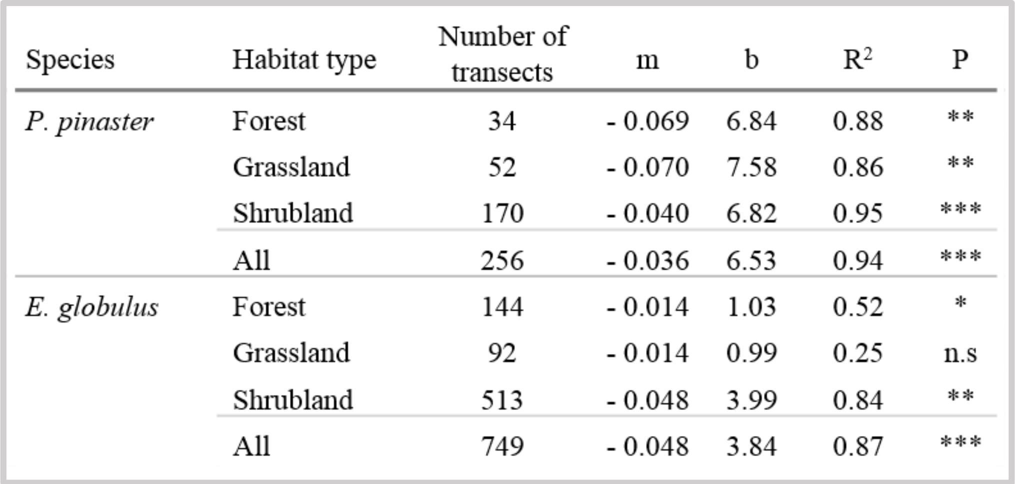
The higher density was registered from plantation boundary to 15 meters away (891.8 seedlings per ha, 35% of the total seedlings registered) (Fig. 2A). In contrast, *E. globulus* seedlings were mostly observed within the boundaries of planted stands, with 102.2 seedlings per ha inside the plantation (60% of the total seedlings). After the plantation boundary, the higher density was observed in the first 15 meters away, with 66.7 seedlings per ha (32% of the total seedlings registered). In *E. globulus* seedlings were observed to a maximum distance of 75m away (with only one seedling registered in this distance from plantation boundary) (Fig.2B). In general, seedling density decreased with the increase of distance from plantation boundary in both species. However, this decrease in seedling density was slighter for *P. pinaster* than for *E. globulus* (Fig.2, 3).

For both species, although clearer for *P. pinaster*, the proportion of old seedlings in relation to young seedlings increased with the distance from plantation edge (and with the decrease of total seedlings density) (Fig.2). Inside the plantation boundary the number of old seedlings was considerable lower than the number of young seedlings, in the both species (17% and 13% of old seedlings for *P.pinaster* and *E. globulus*, respectively) (Fig.2). From the plantation boundary to 15 m further away, the proportion of old seedlings in relation to young seedlings was similar for *P. pinaster* (45% old vs 55% young seedlings) but was considerable lower for *E. globulus* (29% old vs 71% young seedlings) (Fig.2).

Recruitment pattern: seedlings density in relation to distance from plantation

Overall, there was a significant negative relationship between seedlings density and distance from plantation boundary in both study species ($p<0.001$) (Table 2). The highest seedling density occurred close to the plantation boundary (within 15m, inside and outside, of the plantation edge) and rapidly declined until around 30m from the edge, and more slowly farther away (Fig. 3).

TABLE 2. Curve fitting by the negative exponential law (log(y) = a + b (x)) model to observed recruitment curves in *P. pinaster* and *E. globulus* in all sampled transects and in sampled transects per habitat. Average seedlings density y (number per hectare) is a function of distance from plantation edge x. The number of plants is n+1 transformed to avoid zero counts; *m* and *b* are the regression slope and intercept, respectively. n.s. = non-significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.



Species	Habitat type	Number of transects	m	b	R^2	P
P. pinaster	Forest	34	- 0.069	6.84	0.88	**
	Grassland	52	- 0.070	7.58	0.86	**
	Shrubland	170	- 0.040	6.82	0.95	***
	All	256	- 0.036	6.53	0.94	***
E. globulus	Forest	144	- 0.014	1.03	0.52	*
	Grassland	92	- 0.014	0.99	0.25	n.s
	Shrubland	513	- 0.048	3.99	0.84	**
	All	749	- 0.048	3.84	0.87	***

The negative exponential model accounted for a large fraction of variance in seedling density vs distance at both species (R^2 = 0.94 and 0.87 for *P. pinaster* and *E. globulus*, respectively) (Table 2). The regression slope *m* was lower (more negative) for *E. globulus* species (-0.036 seedlings/m for *P. pinaster* vs -0.048 seedlings/m for *E. globulus*) (Table 2), indicating a slower decline in seedling recruitment with distance in *P. pinaster* species (Fig.3). On the other hand, the intercept *b* (ln density at plantation boundary) was considerably higher for *P. pinaster* species (6.53 for *P. pinaster* vs 3.84 for *E. globulus*).

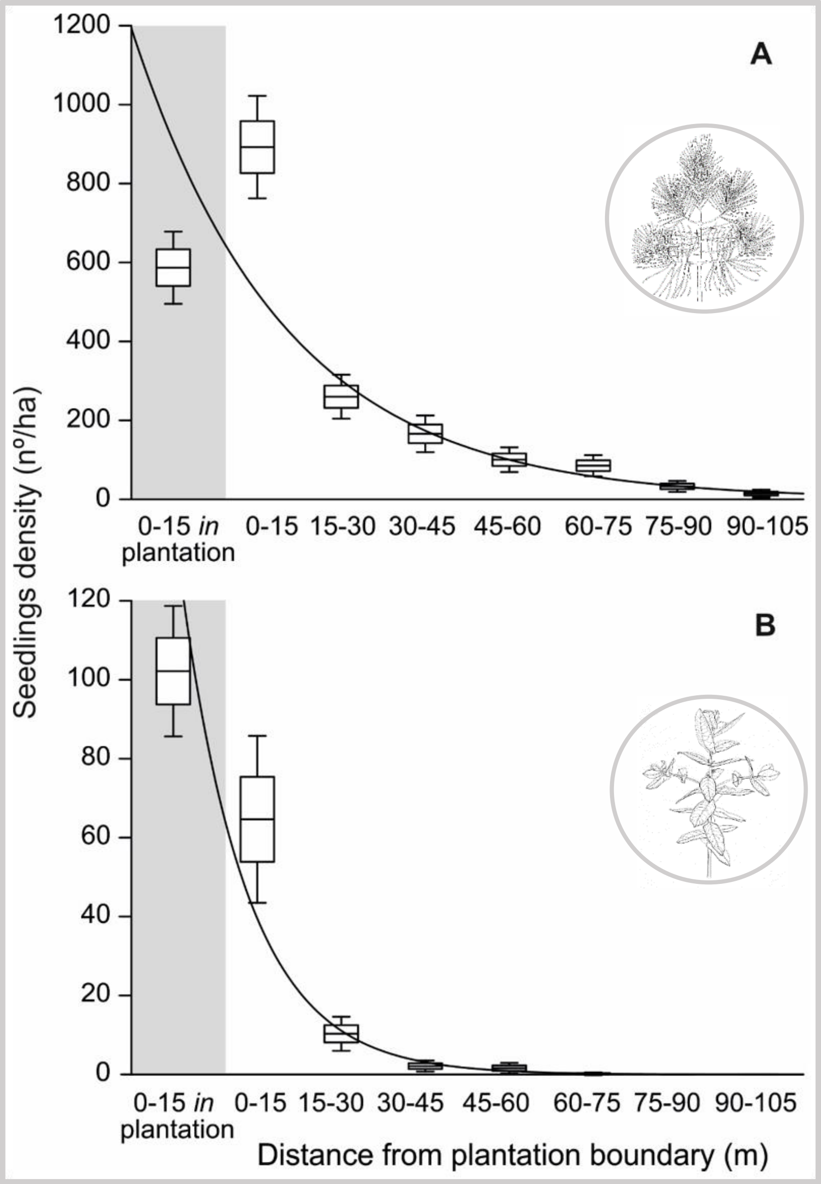


FIGURE 3. Seedlings recruitment curves based on average seedlings density (number per hectare) as a function of the distance from the plantation edge (distance from the edge to the centre of each distance plot). Lines are the fitted negative exponential models. Bars represent the standart error, the middle line represents the average seedling density and lines represent the confidence interval (0.95). **A)** *Pinus pinaster* species and **B)** *Eucalyptus globulus* species. **Note: different scales were used for *P. pinaster* and *E. globulus*.**

The relationship between *P. pinaster* seedlings density and distance from plantation boundary was significant in all habitats adjacent to *P. pinaster* plantations (Forest: $p<0.01$; Grassland: $p<0.01$; Shrubland: $p<0.001$) (Table 2). The negative exponential model accounted for a large fraction of variance in seedling density vs distance at all habitats (Forest: $R^2 = 0.88$; Grassland: $R^2=0.86$; Shrubland: $R^2=0.95$) (Table 2). The lowest regression slope *m* was found in the Shrubland habitat (- 0.040) and the regression slope was similar in the forest and grassland (-0.069 for Forest and -0.070 for Grassland) (Table 2), indicating a slower decline in *P. pinaster* density with distance in the shrubland habitat. The intercept *b* was higher on the Grassland (7.58) and similar between Forest and Shrubland (6.84 and 6.82 in the Forest and Shrubland, respectively) (Table 2).

In the case of *E. globulus*, the relationship between seedlings density and distance from plantation boundary was only significant in the Forest and Shrubland habitats (Forest: $p<0.05$; Grassland: $p=0.144$; Shrubland: $p<0.01$) (Table 2). The negative exponential model accounted for a slight large fraction of variance in seedling density vs distance at only Shrubland habitat (Forest: $R^2 = 0.52$; Grassland: $R^2=0.25$; Shrubland: $R^2=0.84$) (Table 2). The regression slope *m* and the intercept *b* were considerably higher in the shrubland than in the two others habitats (Table 2). This reflect the fact that, in the forest and grassland, the presence of *E. globulus* seedlings was only registered on the firsts 30 m and 15 m from plantation boundary, respectively, and with a relatively low seedling's density (Forest: 3.8 and 0.93 seedlings/ha; Grassland: 5.8 seedlings/ha) (Fig.4 B).

Differences on seedlings density among habitat types

We also analyzed seedlings density with respect to habitat type within each distance plot, and found that seedlings density of both species was higher in Shrublands (*P. pinaster*: n= 170 transect, 1953 seedlings in total; *E. globulus*: n= 513 transects, 377 seedlings in total) (Table 1; Fig. 4).

In both species, Grassland and Forest were the most resistant habitats to seedlings establishment, accounting the lowest densities and with no-presences in the longer distance plots (Table 1; Fig.4). This was more evident in *E. globulus*, with very low values in Grassland (n= 92 transects; registering only 4 seedlings in total and with no presences from 15 m away) and Forest (n=144 transects; registering only 4 seedlings in total and with no presences from 30 m away) (Table 1; Fig.4B). In this species, the effect of habitat on seedlings density was significant until 30 m from the plantation boundary and with Shrubland significantly different from the other two habitats and with no differences between Forest and Grassland (Fig. 4B). From 30 m away the density in the Shrubland was so small (decreasing from 3.1 to 0.25 seedlings/ha at 60-75m) that the differences with the other habitats (without eucalyptus presence in this distance) were non-significant (Fig. 4B).

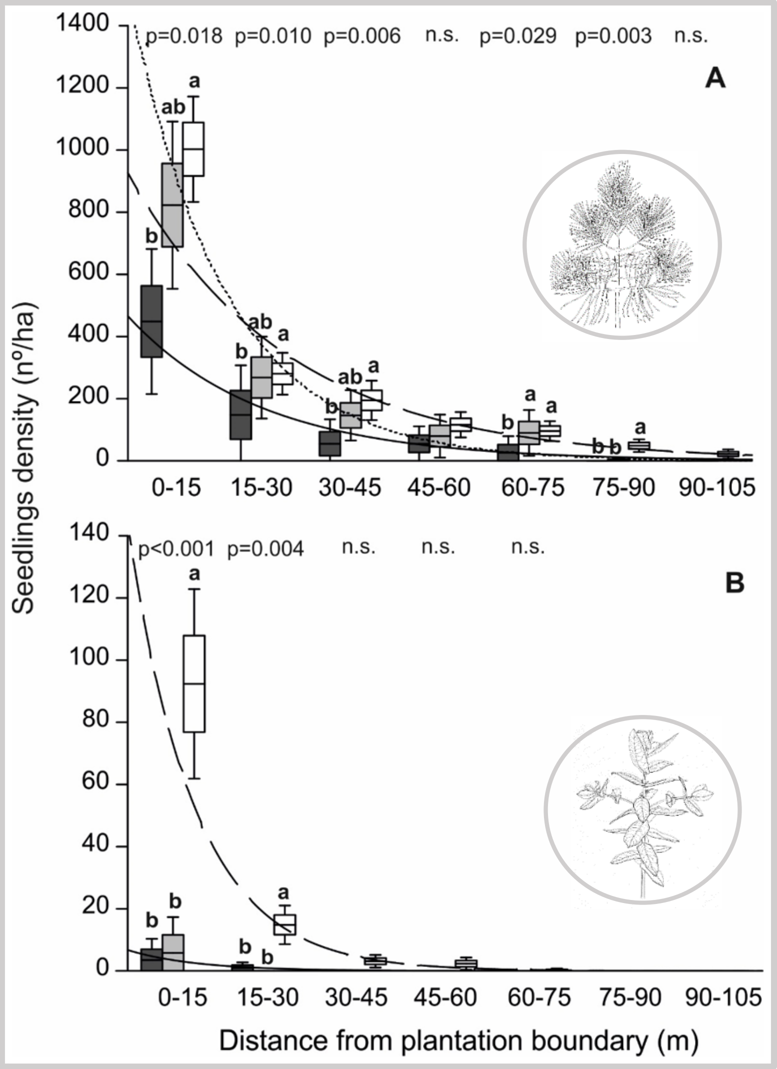


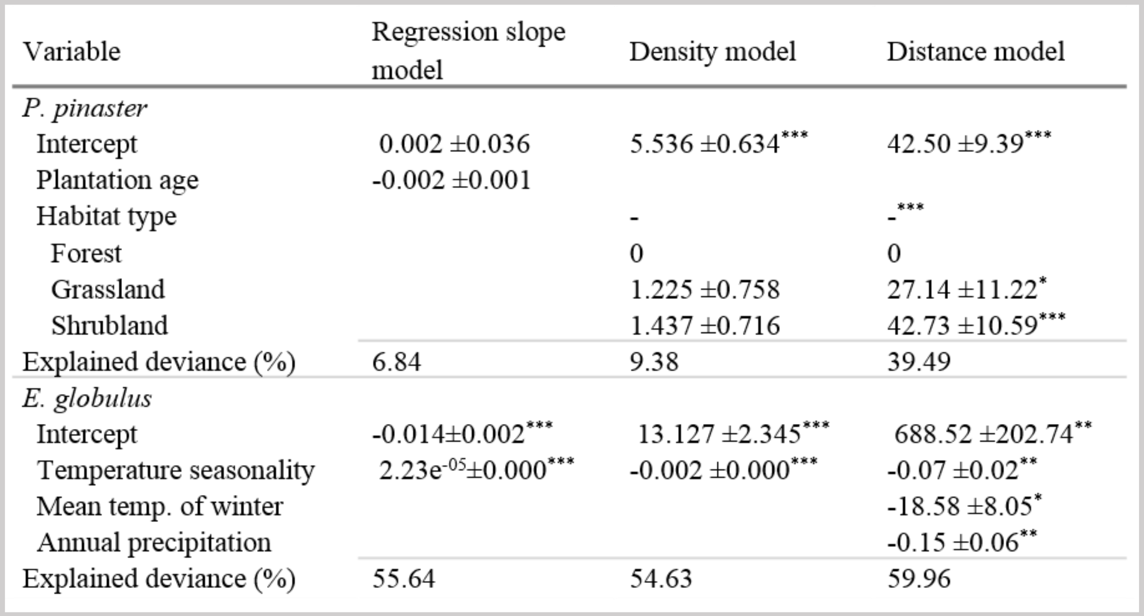
FIGURE 4. Seedlings recruitment curves based on average seedlings density (number per hectare) as a function of distance from the plantation edge (distance from the edge to the centre of each distance plot) in each habitat type. Lines are the fitted negative exponential models for forest (solid line), grassland (small dashed lines) and shrubland (wide dashed lines). Bars represent the standard error, the middle line represent the average seedling density and lines represent the confidence interval (0.95) for forest (dark grey bars), grassland (grey bars) and shrubland (open bars). Different letters represent significant differences among habitat types in each distance plot. P values, according to Kruskal-Wallis ANOVA, are provided if below 0.05. n.s. = non-significant statistically. **A)** *Pinus pinaster* species and **B)** *Eucalyptus globulus* species. **Note: different scales were used for *P. pinaster* and *E. globulus*.**

In *P. pinaster* species the effect of habitat type was only non-significant in the fourth (45-60m) and last (90-105m) distance plots (Fig. 4A). In the last distance plot (90-105 m) we only found *P. pinaster* seedlings in the Shrubland habitat (with an average density of 22 seedlings/ha) (Fig. 4A). The higher densities of *P. pinaster* seedlings were registered in the Shrubland and the lower densities were registered in the Forest habitat. These two habitats were always significantly different (except in the fourth and last distance plots) (Fig. 4A). The Grassland was the second habitat with higher *P. pinaster* seedlings density but only significantly different from Forest in the fifth distance plot (60-75 m) and of Shrubland in the sixth distance plot (75-90) (Fig. 4A).

The factors that influence the 'recruitment curves'

When investigating the factors influencing *P. pinaster* recruitment curves (relationship between seedlings density and distance from plantation) none climatic variable had influence and there only was a significant influence of explanatory variable habitat type in distance model (Table 3).

TABLE 3. Generalized linear models for 'recruitment curve' of *P. pinaster* (N=21) and *E. globulus* (N=16) – the regression slope of the exponential model (regression slope model), the intercept b (ln density at plantation boundary – density model) and maximum distance from plantation boundary with seedlings presence (distance model). For each response variable, explanatory variables kept in the respective final model are indicated through their coefficient ± SE. Significance: ***p < 0.001, **p < 0.01, *p < 0.05.



Variable	Regression slope model	Density model	Distance model
P. pinaster			
Intercept	0.002 ±0.036	5.536 ±0.634***	42.50 ±9.39***
Plantation age	-0.002 ±0.001		
Habitat type		-	-***
Forest		0	0
Grassland		1.225 ±0.758	27.14 ±11.22*
Shrubland		1.437 ±0.716	42.73 ±10.59***
Explained deviance (%)	6.84	9.38	39.49
E. globulus			
Intercept	-0.014±0.002***	13.127 ±2.345***	688.52 ±202.74**
Temperature seasonality	2.23e^{-05}±0.000***	-0.002 ±0.000***	-0.07 ±0.02**
Mean temp. of winter			-18.58 ±8.05*
Annual precipitation			-0.15 ±0.06**
Explained deviance (%)	55.64	54.63	59.96

The main explanatory variable in regression slope model was plantation age, although without a significant influence and only explained 6.84% of the deviance (Table 3). The density model (ln density at plantation boundary) showed that the density was influenced by habitat type (but not significantly), although habitat type only explained 9.38% of the deviance (Table 3). The distance model showed that maximum distance that seedlings can establish was significantly influenced by plantation habitat type, explaining 39.49% of the deviance (Table 3). The seedlings establish a higher distance from plantation boundary in shrubland habitat (Fig.5).

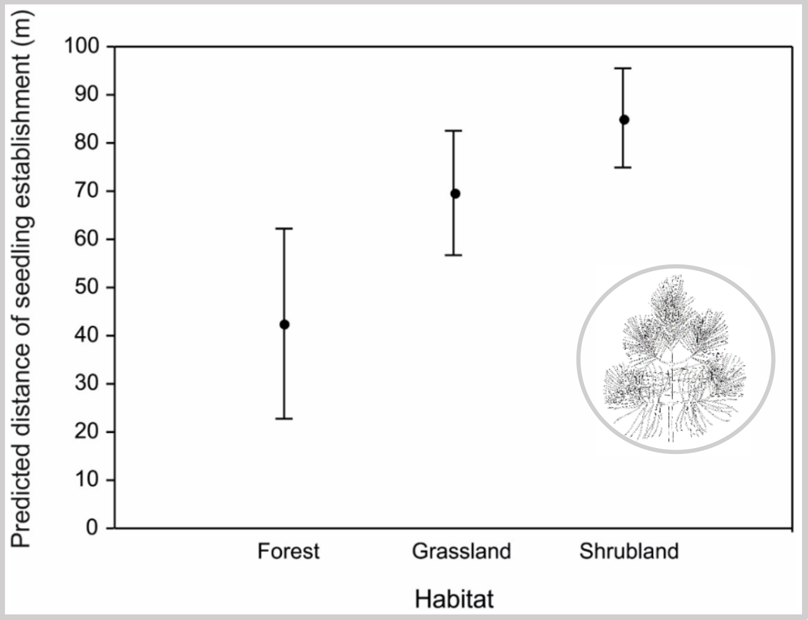


FIGURE 5. Predictions of the maximum distance from plantation boundary with *P. pinaster* occurrence (distance model) in each sampled habitat type (forest, grassland, and shrubland). The point represent predicted distance means ± 95 % confidence intervals.

The *E. globulus* recruitment was only influenced by climatic variables (Table 3). Regression slope model showed that slope of the relationship between seedlings density and distance from plantation was significantly influenced by temperature seasonality, explaining alone 55.56% of the deviance (Table 3). The regression slope was less negative (indicating a slower decline in seedling recruitment with distance) were temperature seasonality was higher, with high temperature seasonality indicative of a large seasonal differences in temperature (pronounced season) (Fig.6a). The main explanatory variables in density model was also temperature seasonality, explaining 54.63% of the deviance (Table 3). The intercept *b* (ln density at plantation boundary) of the recruitment curve model was higher when temperature seasonality was lower (Fig.6b). According to distance model, the seedlings establishment was registered at higher distances from plantation in the sites with lower temperature seasonality, higher winter temperatures and higher precipitation (Fig. 6c-i, c-ii, c-iii). This model explained 59.96% of the deviance (Table 3).

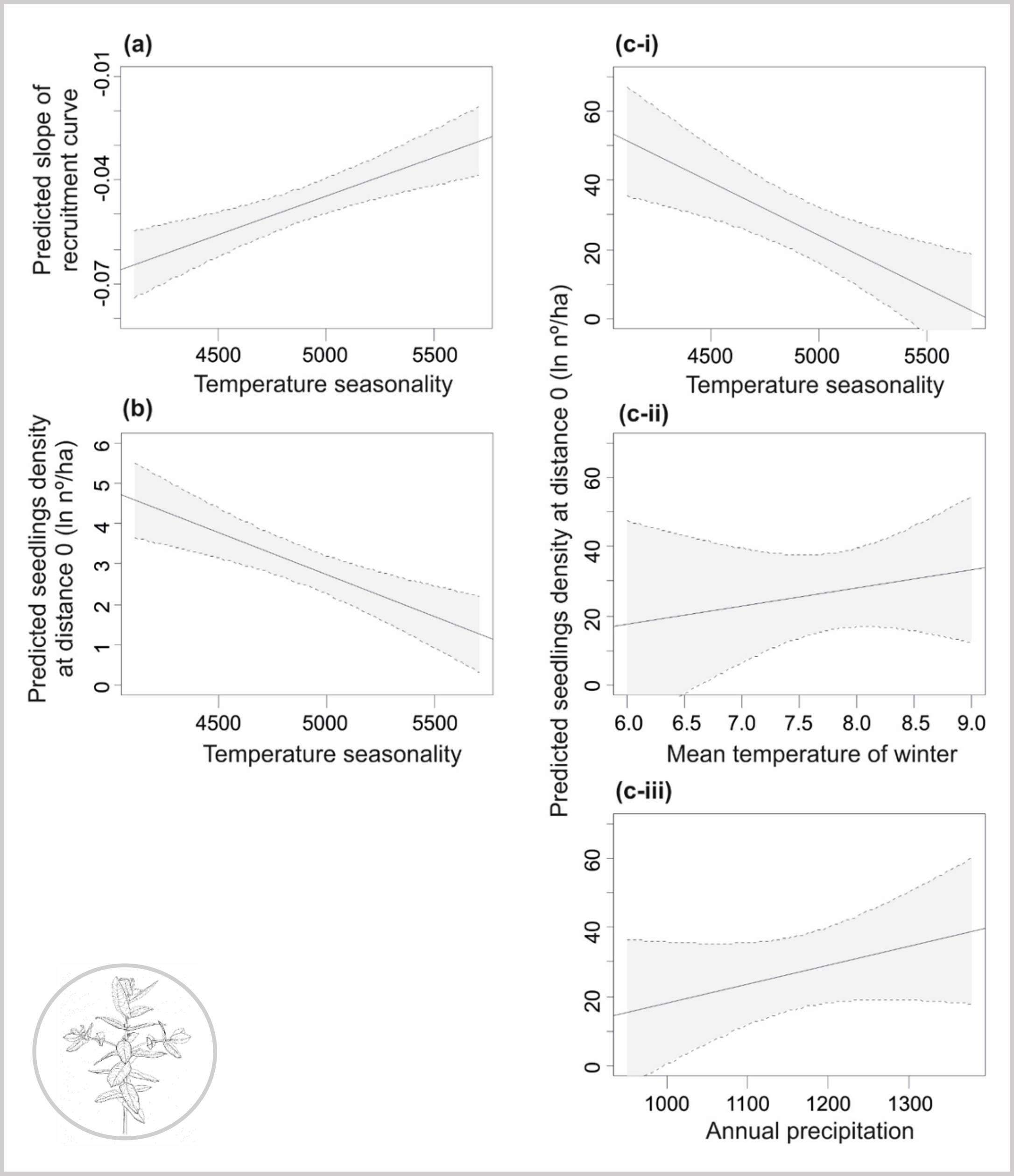


FIGURE 6. Predictions of the significant variables effects on *E. globulus* recruitment: (a) the effect of temperature seasonality on the slope of the recruitment curve; (b) the effect of temperature seasonality on eucalypts density at plantation boundary (c) the effect of the three significant variables [Temperature seasonality (c-i), mean temperature of winter (ºC) (c-ii) and Mean annual precipitation (mm) (c-iii)] on the maximum distance from plantation boundary with *E. globulus* occurrence. The solid lines represent the predicted means and dashed lines are 95% confidence intervals.

DISCUSSION

Overall, we observed as expected that the recruitment of *P. pinaster* and *E. globulus* decreased exponentially from the plantation edge to adjacent habitas. However, species presented very different characteristics of recruitment, regarding the amount of seedlings and the distance of colonization, much higher for *P. pinaster*. Recruitment of both studied species was also influenced by different factors, with *P. pinaster* being independent of climatic variables and only related to habitat type, while *E. globulus* recruitment was mainly related to climate variables.

Spatial patterns of seedling recruitment

Concerning our initial research objective, the results from the recruitment spatial pattern showed that seedling recruitment was higher close to the parent (plantation) in both species. This recruitment pattern followed the Hubbell model (Hubbell, 1980) which proposes that since seed density is very high close to the parent plant, recruitment is higher there, at least in its early stages.

Typically, in wind-dispersed species, seeds rain density decreases as a function of distance from the parent plant (Houles, 1995). The spatial distribution of dispersed seeds of Mediterranean pine species, including *P. pinaster*, have been characterized as leptokurtic, with a peak very close to the source and declining sharply with distance, but with an extended tail of long distance dispersal (Higgins and Richardson, 1999; Rouget and Richardson, 2003). Calviño-Cancela and Rubido-Bará (2013) found that seeds density of *E. globulus* declines sharply with distance from plantation with most seeds falling directly under the canopy. Thus, our results showed that the spatial distribution of *P. pinaster* and *E. globulus* recruits with distance from the source population was similar to the distribution of dispersed seeds (i.e. seed dispersion and seedling recruitment follow the same pattern). This suggests that the probability of a dispersed seed germinating is independent of the distance to the forest edge (Lavi et al., 2005; Nathan et al., 2000).

However, old seedlings proportion increased as a function of distance from plantation in both species. The most well-known hypothesis, distance-dependent coexistence mechanism explained by the Janzen and Connell Hypothesis (Connell, 1971; Janzen, 1970), suggests that specialist enemies (such as pathogens) are frequently the cause of such pattern since they are more likely to damage conspecific seedlings the closer to the parent tree. Intraspecific competition could also explain this pattern. In areas of high conspecific density the intraspecific competition could decrease the survivorship (negative density-dependence hypothesis) (Comita et al., 2014).

The fraction of plants that can establish at long distances have a significant ecological and evolutionary importance on both temporal and spatial scales (Nathan and Muller-Landau, 2000; Nathan et al., 2001). Once established these plants can be expected to function as a new seed source that will disperse seeds further in to the natural environment (Lavi et al., 2005), long-distance dispersal is the main factor determining the expansion rate of a plant population (Cain et al., 2000; Horn et al., 2001; Neubert and Caswell, 2000). The majority of the *E. globulus* recruited seedlings was located less than 15 m from the plantation edge (92% of the total seedlings recorded) and there were very few seedlings at distance 30-60m (21 seedlings in total, 1.9 seedlings/ha) and only one seedling at distance 75m from plantation edge (0.2 seedlings/ha). In *P. pinaster,* there were 79.8 seedlings/ha at distance 30-105m from plantation edge (764 seedlings in total). Thus, the population expansion was substantially more evident in *P. pinaster*, by establishing outlying populations and accelerating the expansion rate.

The two species have very different seed dispersal abilities. *P. pinaster* seeds have large wings as a specialized wind dispersal structure and can be dispersed to long distances. In opposition, *E. globulus* seeds are smaller, with no adaptation for dispersal mechanism (wings or fleshy tissues) and are mainly dispersed within the capsules, and fruit dispersal by wind is presumed to be more limited (Calviño-Cancela and Rubido-Bará, 2013; Rejmánek and Richardson, 2011). Cremer (1977) concluded that *E. globulus* has a limited dispersal ability, with seeds dispersing over quite short distances. Thus, it appears that one of the main constraint for the spread of *E. globulus* outside plantation sites is their limited seed dispersal (Rejmánek and Richardson, 2011; Ritter and Yost, 2009).

Recruitment as a function of habitat suitability

From the findings in our study, studied species seem to differ in establishment success and spread over habitats outside plantation sites, due to differences in their seed dispersal abilities, seedlings establishment success, and adaptability to different habitat types and environmental conditions.

The higher *P. pinaster* recruitment outside plantation (i.e. high proportion of new individuals able to germinate and grow in the natural habitat), the smaller decline in seedlings density with the increasing distance from plantation boundary (smaller slope of recruitment model) and the longer tail of the recruitment curve (longer distance of seedling establishment from plantation sites), demonstrated clearly that *P. pinaster* is more successful colonizing from plantation into different native plant communities than *E. globulus*.

Results from our survey revealed the suitability of all studied habitat types for *P. pinaster* establishment, although their recruitment was more limited in the forests. Since *P. pinaster* is a primary colonizing species and requires a high light regime to grow (Gil et al., 1990), forests are less suitable for their establishment in comparison with open habitats (such as grasslands and shrublands). Other studies also suggest that shrub cover did not compete with *P. pinaster* seedlings (Calvo et al., 2003; Rodríguez-García et al., 2011, 2010, 2007) and under water stress conditions facilitation processes may be more frequent than competition (Rodríguez-García et al., 2010).

Our results indicate that *E. globulus* can establish with some regularity within planted stands, and this suggests that the species is becoming naturalized (*sensu* Richardson et al., 2000) in Portugal. However, like in other studies, the occurrence of natural regeneration was restricted to vicinity of plantations and in low numbers (Callaham et al., 2013; Larcombe et al., 2013; Ritter and Yost, 2009). Thus, there were no indications that *E. globulus* is spreading rapidly, or in large numbers, away from the boundaries of plantations into natural habitats as it is characteristic of invasive species (*sensu* Richardson et al. 2000).

Recently, in Catry et al. (2015) study, eucalypts presence was found in 60 % of the sampled roadside transects adjacent to eucalypt plantations, in Portugal. On the contrary, we only found 13 % of the total plots surveyed outside plantation sites with eucalypts presence. One reason for this discrepancy could be the differences in the vegetation structure and disturbance level between roadsides and natural habitats. Roadside is a habitat with particular characteristics with high human disturbance and high percentage of soil without vegetation. These characteristics seem to increase the suitability to eucalypts establishment, increasing the window of opportunity for eucalypt seeds to reach and colonize new areas (da Silva et al., 2011; Lorentz and Minogue, 2015; Rejmánek and

Richardson, 2011). Thus, our results suggest that eucalypts establishment is limited in the natural habitats outside managed plantation sites, compared with values recorded in the roadsides (Catry et al., 2015). This is particularly true when the habitat types surrounding the sampled plantations are forests or grasslands. *E. globulus* has been classified as shade-intolerant species, requiring a high light regime to establish (Correia et al., 1989). It´s known that native forests dominated by pedunculated oak, as other broad leaved trees, have low light irradiance regimes at ground level compared to open vegetation of shrubland (Barbier et al., 2008). Therefore, the lowest probability of eucalypts occurrence found in forest patches is likely to be caused by a negative shading effect. Likewise, Calviño-Cancela and Rubido-Bará (2013) showed lower *E. globulus* seedling emergence under native forest patches (pedunculated oak) than in the shrubland. In addition, the survival of eucalypts seedlings is compromised in dense vegetation sites (Rejmánek and Richardson, 2011). Grasslands normally show dense vegetation (and low ground surface heterogeneity) and high seasonal fluctuation in water soil and nutrients availability (Rundel et al., 2014). These factors could negatively affect eucalypts seedling survival (Rejmánek and Richardson, 2011). Therefore, the window of opportunity for eucalypts seeds to reach suitable sites in grasslands and forests are rather narrow.

Environmental features and recruitment success

Others factors that can limit eucalypts colonization are related with specific requirements to seed germination and high mortality during initial phase of seedling growth, mainly due to unsuitable environmental conditions or competition with other plants (Callaham et al., 2013; da Silva et al., 2011; Rejmánek and Richardson, 2011). Eucalypts produce very large quantities of seeds but those seeds are very small and present no obvious endosperm. Thus the newly emerged seedlings (sustained by cotyledon photosynthesis) need that their roots penetrate into suitable wet substrate very quickly (Rejmánek and Richardson, 2011). As a result, water deficit negatively affects *E. globulus* seed germination and establishment (Humara et al., 2002; López et al., 2000). Therefore, water availability and frost occurrence are known to affect *E. globulus* recruitment, seedling establishment, and survival. It´s well known that water deficit can compromise summer survival of young eucalypts (Jacobs, 1955; Stoneman, 1994; Stoneman et al., 1994) and frost may directly kill foliage and buds, and sometimes the whole plant (Cremer et al., 1984). These facts may explain why *E. globulus* recruitment was higher in sites with lower temperature seasonality and why sites with higher winter temperatures, higher annual precipitation and lower seasonal differences in temperature are more likely to have *E. globulus* establishment at longer distances from plantation boundary. Previous studies (i.e. Águas et al., 2014; Catry et al., 2015; Larcombe et al., 2013) pointed at the same direction. Larcombe et al. (2013) found that sites where precipitation seasonality was higher (existence of a rainfall season and accentuated water stress in late summer) had lower probability to have natural establishment of this species, in Australia.

Águas et al (2014) and Catry et al (2015), showed that *E. globulus* recruitment is more abundant in areas with higher annual precipitation, lower thermal amplitude, and with lower number of frost days, in Portugal. Catry et al. (2015) found that eucalypts density was lowest below c. 600 – 700 mm of annual precipitation and then sharply increased up to around 1500 mm. They also found that eucalyptus density reached the lowest values above 2400 mm. The annual precipitation sampled in our study was 1103 mm in average (ranging from 636 mm to 1438 mm), which seems to be the optimal for eucalypts establishment. Studies also indicate that water availability from precipitation is the most limiting factor for *P. pinaster* recruitment (Rodriguez-Garcia et al., 2011; Rodríguez-García et al., 2010; Ruano et al., 2009). However, *P. pinaster* Iberian populations show elevated levels of genetic variability that has enable local adaptation to ecological conditions (Alía et al., 1996) and is known that this species has enormous ecological plasticity, tolerates conditions varying from Mediterranean to Temperate-Oceanic climates (Rodríguez-García et al., 2010). These facts could explain why climate variables did not influence *P. pinaster* recruitment in our study. The climate range among sites sampled in our study could have been insufficient to affect *P. pinaster* recruitment. Only habitat type was an important factor to determine the maximum distance from plantation edge with pine presence, with lower distances registered in forests. This was expected because the presence of other trees can physically limit the dispersal distance of wind-dispersed seeds.

Onwards (management)

Regarding our study objectives, our surveys were undertaken predominantly along treeless habitats surrounding plantations (grasslands and shrubland) with high conservation value. The colonization of these habitats by trees, both native and non-native trees, has profound consequences for community structure, biodiversity, and ecosystem functioning (Richardson and Rejmánek, 2011; Rundel et al., 2014). Our study showed how is the spatial spread pattern of *P. pinaster* and *E. globulus* from plantation into adjacent habitats. It is important to consider the spatial dynamics of landscape patches for better management of landscape characteristics such as connectivity or permeability in relation to movement of key species (such as planted species) (Hobbs et al., 2014). Hobbs et al. (2014) advanced the idea of "Managing the whole landscape" in situations where we need to deal with mixtures of natural ecosystems that are substantially altered by humans. Following their idea, researches as our study have crucial importance for ensure landscape functionality.

Alterations in ecosystem structure and function are one of the most reported effects of pine colonization into treeless areas, particularity in southern hemisphere (Higgins and Richardson, 1998). This species can show continuous recruitment with regeneration pulses over time (Rodríguez-García et al., 2011) and in Portuguese managed plantations the final cut of *P. pinaster* trees occurs around 45 years after plantation, so their ability to develop significant propagule pool is high. Considering these facts and the current results (the suitability of grasslands and shrubland for *P. pinaster* recruitment and the high proportion of old seedlings at long distances from the plantations) we can conclude that the colonization of *P. pinaster* into grasslands and shrublands can lead to a conversion of such treeless systems to pine forests if no control and monitoring practices of natural regeneration outside plantations are implemented. Indeed, the spread of pines outside plantation sites should also be a concern, whether encroaching from forests in the native range or from plantations in the introduced range (Taylor et al., 2016). However, the control and monitoring practices could be difficult to implement because of the low managements requirements and consequently low human intervention in *P. pinaster* stands. In opposition, industrial *E. globulus* plantations are grown with very short rotations (rotation time in Portugal is 10-12 years) and with a regular basis management, so there are likely to be multiple opportunities for detection and control over the lifetime of a plantation. In addition, limited dispersal ability of *E. globulus* resulted in a localized recruitment close to plantation boundaries which make the implementation of simple management practices, such as maintaining clear firebreaks surrounding the stands, very efficient and would likely greatly reduce likelihood of establishment and their spread outside plantation sites. Considering the differences in spatial pattern of recruitment between species and the different plantation management, we can conclude that the risk of colonization by *E. globulus* will likely be managed more effectively than that of *P. pinaster*. Moreover, the low residence time of *E. globulus* plantations also contribute to diminish their ability to develop a significant propagule pool. Because of these plantation management and age-related barriers to colonization, it´s important to understand what happens with plantations that are left unmanaged and unharvested (abandoned plantations), where these barriers can disappear. In our study we did not considered sites with accentuated slopes, however both *P. pinaster* and *E. globulus* plantations near drainage lines or stepper slopes need an increased attention because the potential dispersal distances and the difficulty to implement control practices increase in these situations.

We suggest the establishment of "sentinel sites" using remote sensing tools (e.g. Google Earth) as described by Visser et al. (2014). This monitoring system can be used to monitor tree expansion into habitats outside plantation areas, and could, over time, produce very valuable data to improve our understanding of colonization dynamics of these species and could also alert managers to situations where some form of management intervention is needed.

CONCLUSIONS

Nowadays, the expansion of trees into habitats outside plantations is a concern for managers and conservationists. In our study we showed that these forestry plantations have different spatial dynamics. It is important to consider these dynamics (that can lead to a negative connectivity among plantation patches and natural patches, such as invasion) in order to prevent the transition of these species in to adjacent habitats, that ultimately drive ecosystems to stable novel state (Hobbs et al., 2014).

In the case of *E. globulus*, the current study suggested that the colonization outside plantation will likely be remarkably slow when compared to *P. pinaster* (species with long-distance seed dispersal). The difference in seed dispersal ability between species is reflected in their spread patterns. The suitability of all habitats for *P. pinaster* natural regeneration also showed their higher ecological plasticity.

The localized recruitment and lower levels of establishment of *E. globulus* from managed plantations suggested that currently, this species does not demonstrate an invasive behavior. However, future research in abandoned eucalyptus plantation is needed to understand the role of plantation management and age (residence time) as barriers to *E. globulus* colonization.

Based on models results, it is not clear how *P. pinaster* recruitment is affected by climatic conditions. On the other hand, the recruitment of *E. globulus* seedlings was mainly affected by temperature seasonality and recruitment was found to be higher in areas with lower seasonal differences. Based on that, *E. globulus* seedlings establishment showed a more sensitive response to climatic variations than *P. pinaster* species. Considering future climatic changes for the Mediterranean basin, with intensification of dry conditions (increase of maximum summer temperatures and decrease of annual precipitation) and accentuated seasonal temperatures differences (IPCC, 2007), natural recruitment (seedlings survival) of *E. globulus* will likely become harder.

Current results may contribute to increase the knowledge about expansion capability of these species, and may help managers to improve and prioritize eventual control plans in sites with higher suitability to recruitment.

ACKNOWLEDGEMENTS

This research was funded by *Fundação para a Ciência e a Tecnologia (FCT)* and *the Navigator Company* in the frame of Patrícia Fernandes PhD scholarship from FCT (SFRH/BDE/51709/2011); Pedro Pinho was supported by a postdoctoral Grant from FCT (SFRH/BPD/75425/2010). We would like to thank the Navigator Company for providing access to plantations, maps and data. Namely, we would like to thank: André Carvalho, Eduardo Mendes, Luís Alarico, João Bandeira, Américo Campos, Francisco José Inácio, Isidro Costa, João Pires, Joaquim Fonseca, Jorge Lourenço, José Carlos Pereira, Leonel Almeida and Ricardo Mendes. We express our gratitude to Sara Coelho, Elsa Gonçalves, Andreia Anjos and Márcia Vieira for the help collecting data during field surveys. We also thank David Richardson and an anonymous reviewer for their valuable comments.

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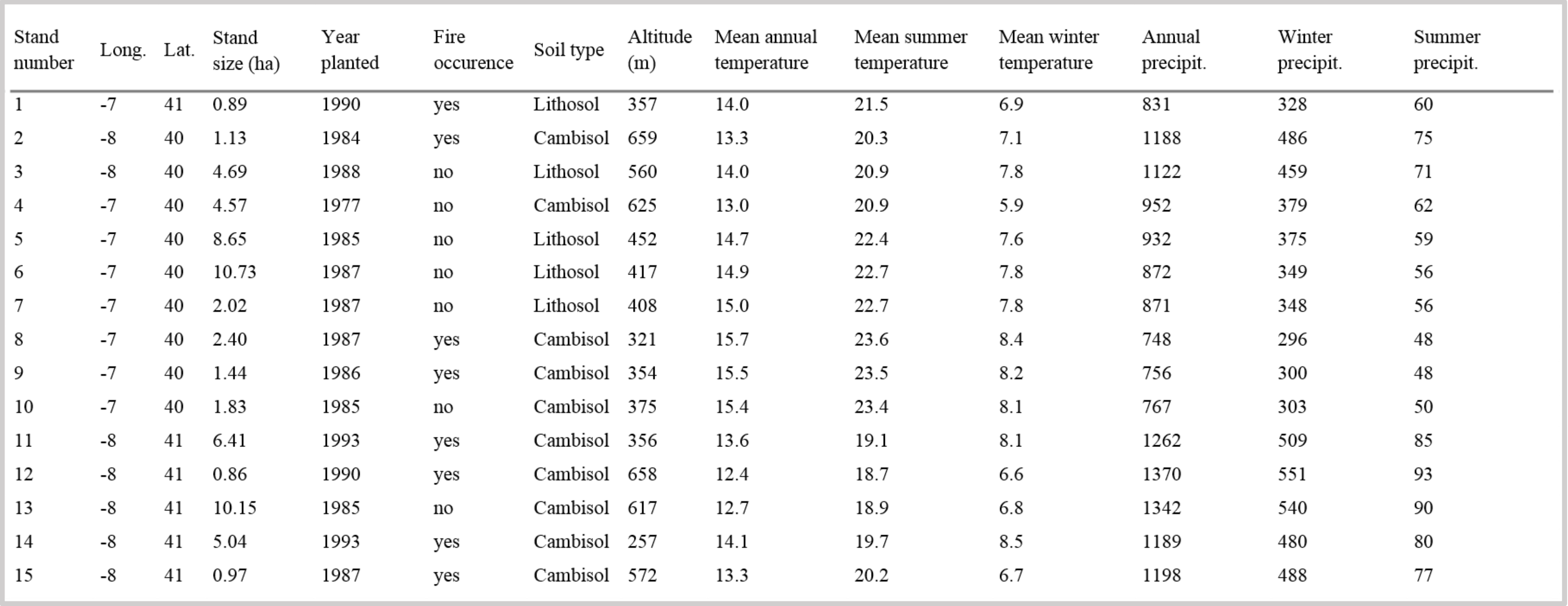
APPENDIX

TABLE A1. Characteristics of ***Pinus pinaster*** sites and stands sampled in this study.



Stand number	Long.	Lat.	Stand size (ha)	Year planted	Fire occurence	Soil type	Altitude (m)	Mean annual temperature	Mean summer temperature	Mean winter temperature	Annual precipit.	Winter precipit.	Summer precipit.
1	-7	41	0.89	1990	yes	Lithosol	357	14.0	21.5	6.9	831	328	60
2	-8	40	1.13	1984	yes	Cambisol	659	13.3	20.3	7.1	1188	486	75
3	-8	40	4.69	1988	no	Lithosol	560	14.0	20.9	7.8	1122	459	71
4	-7	40	4.57	1977	no	Cambisol	625	13.0	20.9	5.9	952	379	62
5	-7	40	8.65	1985	no	Lithosol	452	14.7	22.4	7.6	932	375	59
6	-7	40	10.73	1987	no	Lithosol	417	14.9	22.7	7.8	872	349	56
7	-7	40	2.02	1987	no	Lithosol	408	15.0	22.7	7.8	871	348	56
8	-7	40	2.40	1987	yes	Cambisol	321	15.7	23.6	8.4	748	296	48
9	-7	40	1.44	1986	yes	Cambisol	354	15.5	23.5	8.2	756	300	48
10	-7	40	1.83	1985	no	Cambisol	375	15.4	23.4	8.1	767	303	50
11	-8	41	6.41	1993	yes	Cambisol	356	13.6	19.1	8.1	1262	509	85
12	-8	41	0.86	1990	yes	Cambisol	658	12.4	18.7	6.6	1370	551	93
13	-8	41	10.15	1985	no	Cambisol	617	12.7	18.9	6.8	1342	540	90
14	-8	41	5.04	1993	yes	Cambisol	257	14.1	19.7	8.5	1189	480	80
15	-8	41	0.97	1987	yes	Cambisol	572	13.3	20.2	6.7	1198	488	77

Summer months: July, August, September; *Winter months*: December, January, February
Long.=longitude; Lat.=latitude; Precipit.=precipitation

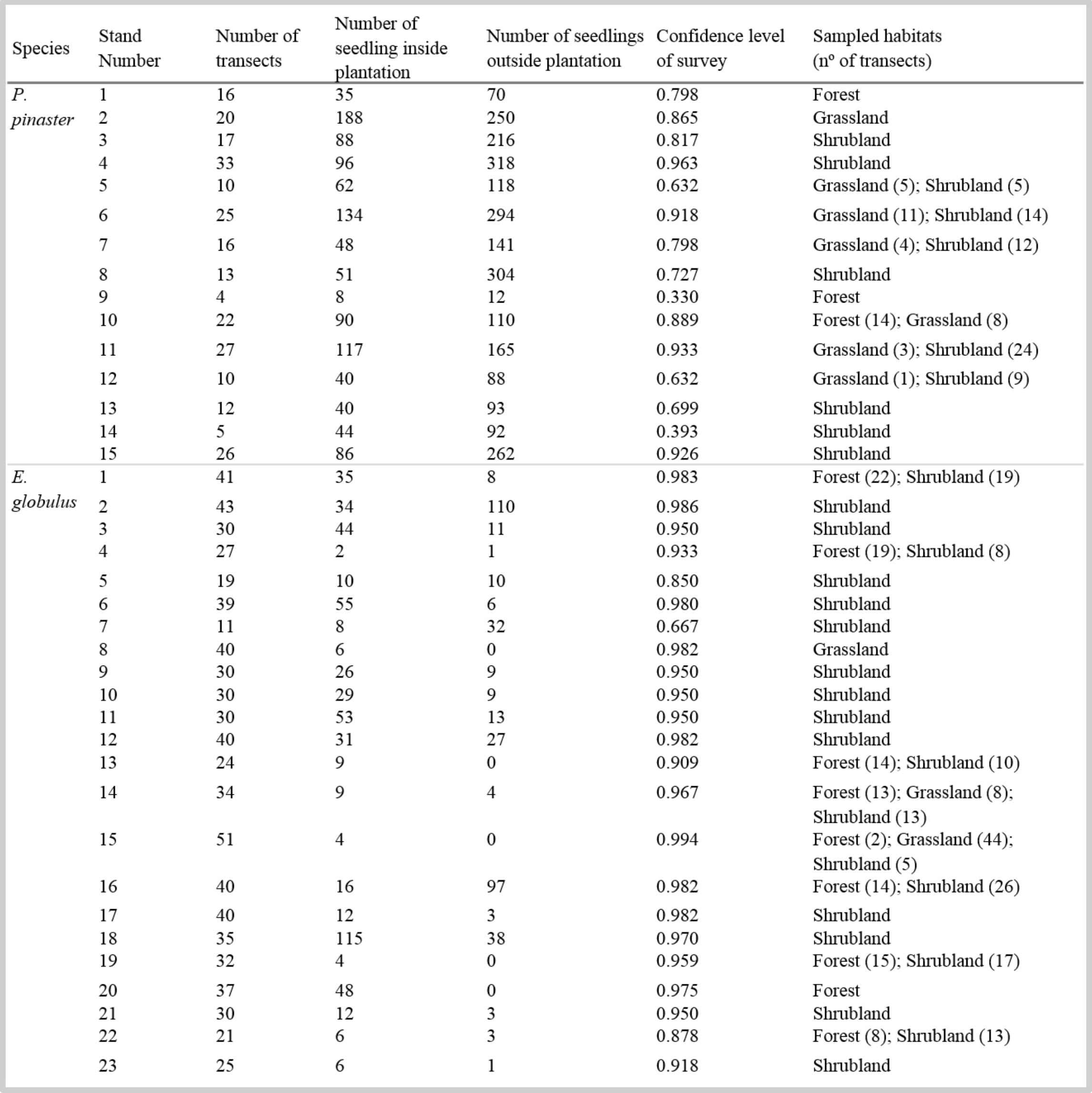
TABLE A2. Characteristics of ***Eucalyptus globulus*** sites and stands sampled in this study.

Stand number	Long.	Lat.	Stand size (ha)	Year planted	Fire occurence	Soil type	Altitude (m)	Mean annual temperature	Mean summer temperature	Mean winter temperature	Annual precipit.	Winter precipit.	Summer precipit.
1	-8	41	0.95	1998	yes	Cambisol	373	13.90	20.2	7.7	1209	479	93
2	-8	41	1.05	1998	yes	Cambisol	373	14.00	19.6	8.2	1191	472	92
3	-8	41	1.90	1998	yes	Cambisol	348	13.90	19.3	7.2	1209	479	93
4	-8	41	0.45	1998	yes	Cambisol	261	14.00	19.9	8.0	1194	473	92
5	-8	41	1.35	2003	yes	Cambisol	190	14.70	21.2	6.2	1073	422	85
6	-8	41	5.38	2002	yes	Cambisol	551	12.60	22.7	7.8	1378	550	108
7	-8	41	0.66	2002	yes	Cambisol	438	12.60	23.9	9.0	1378	550	108
8	-8	41	1.19	2000	no	Cambisol	706	11.60	21.7	9.4	1438	581	105
9	-8	41	5.67	2001	yes	Cambisol	127	14.30	20.7	6.2	1172	472	94
10	-8	41	8.28	2000	yes	Cambisol	204	13.80	19.8	9.2	1231	495	97
11	-8	41	4.79	2003	yes	Cambisol	658	13.10	22.7	7.8	1291	519	93
12	-8	41	0.76	1998	yes	Cambisol	365	13.90	20.3	7.8	1200	483	88
13	-7	40	4.57	1987	no	Lithosol	671	13.30	20.9	7.8	927	369	60
14	-7	40	7.72	2002	no	Lithosol	388	15.00	20.2	7.7	871	348	56
15	-7	40	11.64	2001	no	Lithosol	288	16.10	20.3	7.8	792	317	50
16	-7	40	3.48	2002	no	Lithosol	311	15.30	22.6	8.2	938	384	59
17	-7	40	1.89	2000	yes	Cambisol	658	13.10	21.9	8.1	1075	435	70
18	-8	41	5.44	2000	no	Cambisol	146	14.60	21.2	8.4	1099	442	84
19	-7	41	6.44	1992	no	Lithosol	163	15.10	22.4	8.3	636	247	47
20	-8	40	3.30	2001	no	Lithosol	560	14.00	18.6	7.0	1122	459	71
21	-8	40	3.18	1999	yes	Lithosol	377	15.00	18.6	7.0	951	386	60
22	-8	40	3.32	2001	yes	Lithosol	437	14.70	18.2	5.6	1021	415	65
23	-8	40	1.61	1999	yes	Lithosol	372	15.10	20.0	8.6	972	394	62

Summer months: July, August, September; *Winter months*: December, January, February

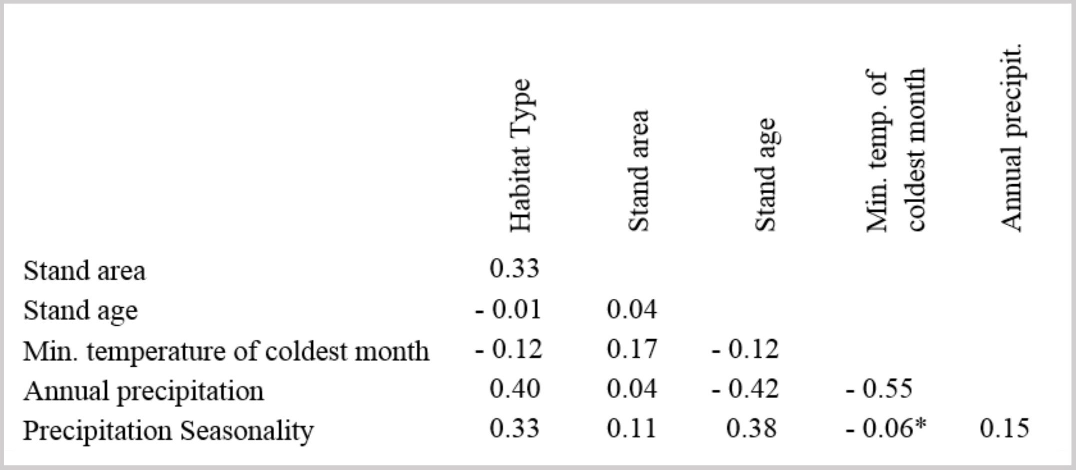
Long.=longitude; Lat.=latitude; Precipit.=precipitation

TABLE A3. Sampling distribution across sampled stands and habitats. Also shown the level of confidence (based on transect number) that would encounter *P. pinaster* and *E. globulus* seedlings if they were present at a particular site (see field sampling method text for details how confidence level is calculated).



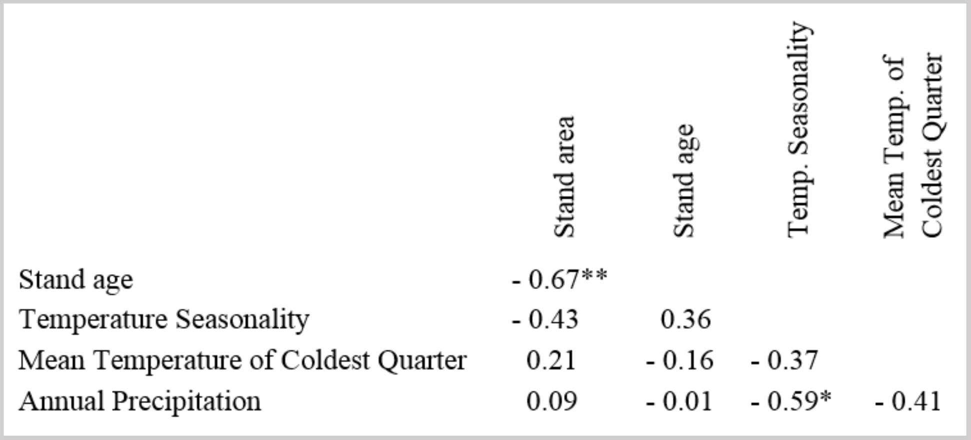
Species	Stand Number	Number of transects	Number of seedling inside plantation	Number of seedlings outside plantation	Confidence level of survey	Sampled habitats (nº of transects)
P. pinaster	1	16	35	70	0.798	Forest
	2	20	188	250	0.865	Grassland
	3	17	88	216	0.817	Shrubland
	4	33	96	318	0.963	Shrubland
	5	10	62	118	0.632	Grassland (5); Shrubland (5)
	6	25	134	294	0.918	Grassland (11); Shrubland (14)
	7	16	48	141	0.798	Grassland (4); Shrubland (12)
	8	13	51	304	0.727	Shrubland
	9	4	8	12	0.330	Forest
	10	22	90	110	0.889	Forest (14); Grassland (8)
	11	27	117	165	0.933	Grassland (3); Shrubland (24)
	12	10	40	88	0.632	Grassland (1); Shrubland (9)
	13	12	40	93	0.699	Shrubland
	14	5	44	92	0.393	Shrubland
	15	26	86	262	0.926	Shrubland
E. globulus	1	41	35	8	0.983	Forest (22); Shrubland (19)
	2	43	34	110	0.986	Shrubland
	3	30	44	11	0.950	Shrubland
	4	27	2	1	0.933	Forest (19); Shrubland (8)
	5	19	10	10	0.850	Shrubland
	6	39	55	6	0.980	Shrubland
	7	11	8	32	0.667	Shrubland
	8	40	6	0	0.982	Grassland
	9	30	26	9	0.950	Shrubland
	10	30	29	9	0.950	Shrubland
	11	30	53	13	0.950	Shrubland
	12	40	31	27	0.982	Shrubland
	13	24	9	0	0.909	Forest (14); Shrubland (10)
	14	34	9	4	0.967	Forest (13); Grassland (8); Shrubland (13)
	15	51	4	0	0.994	Forest (2); Grassland (44); Shrubland (5)
	16	40	16	97	0.982	Forest (14); Shrubland (26)
	17	40	12	3	0.982	Shrubland
	18	35	115	38	0.970	Shrubland
	19	32	4	0	0.959	Forest (15); Shrubland (17)
	20	37	48	0	0.975	Forest
	21	30	12	3	0.950	Shrubland
	22	21	6	3	0.878	Forest (8); Shrubland (13)
	23	25	6	1	0.918	Shrubland

TABLE A4. Pearson's correlation coefficients among the predictors used to model *P. pinaster* 'recruitment curve'. Significance: $p<0.01$ **; $p<0.05$ *.



	Habitat Type	Stand area	Stand age	Min. temp. of coldest month	Annual precipit.
Stand area	0.33				
Stand age	- 0.01	0.04			
Min. temperature of coldest month	- 0.12	0.17	- 0.12		
Annual precipitation	0.40	0.04	- 0.42	- 0.55	
Precipitation Seasonality	0.33	0.11	0.38	- 0.06*	0.15

TABLE A5. Pearson's correlation coefficients among the predictors used to model *E. globulus* 'recruitment curve'. Significance: $p<0.001$ ***; $p<0.01$ **; $p<0.05$ *.

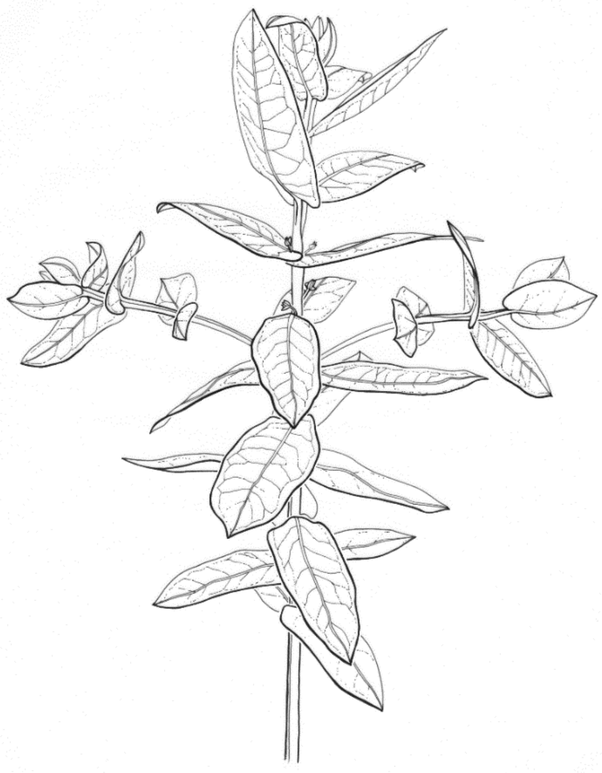


	Stand area	Stand age	Temp. Seasonality	Mean Temp. of Coldest Quarter
Stand age	- 0.67**			
Temperature Seasonality	- 0.43	0.36		
Mean Temperature of Coldest Quarter	0.21	- 0.16	- 0.37	
Annual Precipitation	0.09	- 0.01	- 0.59*	- 0.41

CHAPTER 3

WHAT DRIVES *EUCALYPTUS GLOBULUS* NATURAL ESTABLISHMENT OUTSIDE PLANTATIONS?

THE RELATIVE IMPORTANCE OF **CLIMATE**, **PROPAGULE PRESSURE**, AND **SITE CHARACTERISTICS**



Fernandes, P., Máguas, C., Correia, O. & González-Moreno, P. What drives *Eucalyptus globulus* natural establishment outside plantations? The relative importance of climate, propagule pressure, and site characteristics. *Submitted to Biological Invasions*.

ABSTRACT

Eucalyptus globulus has great economic importance in the Iberian Peninsula and it is now the most widespread tree species in Portuguese mainland. We aimed to evaluate the establishment capacity of *E. globulus* from plantations into natural habitats and to understand its association with climate, propagule pressure and host site characteristics. We surveyed 50 *E. globulus* plantations across Portugal looking at *E. globulus* natural establishment (i.e. occurrence and density) in several habitats adjacent to plantations. Eucalypts presence was only recorded in 8% of the plots. Site characteristics (such as habitat type, vegetation cover, and disturbance) were the most important group of variables influencing *E. globulus'* natural recruitment in comparison to climate or propagule pressure variables. Forest and grassland were the most resistant habitats to eucalyptus invasion while highly disturbed areas can be considered hubs for eucalypt density. The high importance of site characteristics reflects that most of the variability in eucalypt establishment occurs at small spatial scales. Thus, monitoring and management efforts should focus on those sites with higher establishment probability (i.e. open areas) trying to promote native vegetation and reduce disturbance levels.

INTRODUCTION

Planted forest accounts for 264 million hectares (7% of the total forest area worldwide) and approximately 25% of which consists of exotic species (FAO, 2010). The use of exotic species in forest plantations (mostly pine and eucalypt) is primarily related to the wide range of ecological requirements of these species, their rapid growth and high productivity. In many countries, these species play a significant role in their economy and are the best choice to achieve high production, especially in areas were native species do not perform well (Dodet and Collet, 2012; Richardson, 1998). However, the prolific nature of the exotic tree species selected in forestry and their widespread use increase the risk of these species to invade natural and semi-natural habitats surrounding plantations (Dodet and Collet, 2012; Essl et al., 2010; Richardson and Rejmánek, 2011; Richardson, 1998; Van Der Meer et al., 1999). Forestry plantations are one of the main sources of plant invaders worldwide (Richardson, 1998). Nowadays, the expansion of exotic trees into habitats outside plantations is a relevant concern for managers and conservationists (Dodet and Collet, 2012; Richardson, 1998). In order to contribute to a sustainable forest management (minimizing ecological risks and maintaining economical productivity) it is important to study the main factors that drive exotic plant species natural establishment and determine the capacity to colonize outside the planted areas (Richardson and Rejmánek, 2011).

Eucalyptus species are among the most planted forest species worldwide. They are mainly planted in temperate, tropical and subtropical regions, where they cover c. 20 million ha (Rejmánek and Richardson, 2011). *Eucalyptus globulus* Labill. (Tasmanian blue gum), native to southeast Australia, is nowadays one of the most important pulpwood species planted worldwide (among other uses), with the main concentration of plantations occurring in the Iberian Peninsula (Potts et al., 2004). This species has a great economic importance in the western region of the Iberian Peninsula (Potts et al., 2004) and it is currently the most widespread tree species in Portuguese mainland, representing 26% of its forest cover (ICNF, 2013). This species was introduced to Portugal in the middle of the 19th century and since the middle of 20th century the development of the pulp and paper industries originated the expansion of *E. globulus* plantation in the country (Alves et al., 2007).

E. globulus has been reported as invasive in seven regions globally: North, Central and South America, Europe, New Zealand, Pacific Islands and Indian Oceans Islands (Rejmánek and Richardson 2013). The invasion risk of *E. globulus* has been identified as “High” in several publications based on the Australian Weed Risk Assessment (Daehler et al., 2004; Gassó et al., 2009; Gordon et al., 2012; Marchante et al., 2014). Weed risk assessments (WRA) are mainly based on species biological traits and expert knowledge (Pheloung et al., 1999), and rarely based on field quantitative information (distribution of the species in the introduced range). On the other hand, eucalypts has been reported as a species with a low invasive risk based on field quantitative studies (Callaham et al., 2013; da Silva et al., 2011; P. Fernandes et al., 2016; Larcombe et al., 2013; Lorentz and Minogue, 2015). In general, eucalypts have rarely spread considerable distances from planting sites, their regeneration is sporadic (Callaham et al., 2013; P. Fernandes et al., 2016; Larcombe et al., 2013; Rejmánek and Richardson, 2011), and have high mortality of seedlings (Calviño-Cancela and Rubido-Bará, 2013; da Silva et al., 2011; Lorentz and Minogue, 2015; Rejmánek and Richardson, 2011). These factors seem to be major reasons for the limited invasiveness of eucalyptus (Rejmánek and Richardson, 2011). Studies on *Pinus* and *Acacia* have found that species invasiveness is related to repeated introductions, planting intensity and long residence time (Gibson et al., 2011; Procheş et al., 2012; John R.U. Wilson et al., 2009), which are the characteristics of many *Eucalyptus* species (including *E. globulus* in Portugal). However, Eucalypts are generally viewed as markedly less invasive than several other widely cultivated trees (e.g. pines and acacias; Rejmánek and Richardson, 2011). Rejmánek and Richardson (2011) opened the question: “Are eucalyptus inherently less invasive, or are they just a ticking time bomb?”. Understanding the factors driving the natural establishment of *E. globulus* should help to evaluate the invasive potential of this species (Richardson and Rejmánek, 2011).

Among the abiotic factors that determine biological invasions, the effect of climate is probably the most studied (Thuiller et al., 2005). Climate has been considered as the main driver of plant distribution from continental to regional scales (Milbau et al., 2009a; Pearson et al., 2004). Indeed, drought summer in the Mediterranean region is highly limiting for plant performance and prevent the establishment success of many exotic plants (Chytrý et al., 2008b). Although *E. globulus* is adaptable to a wide range of environmental conditions, water availability and low temperatures (mainly occurrence of temperatures below 0ºC) are the main limiting climatic factors to their growth in Portugal

(Almeida et al., 1994; Alves et al., 2012; Catry et al. 2015). Thus, given the Mediterranean character of the most of the Portuguese territory (with associated drought stress during summer months), we hypothesise that *E. globulus* establishment is primarily affected by precipitation.

The establishment success of an exotic species depends on the characteristics of the new species itself and the host community, and on the interaction between them (Lonsdale, 1999; Rejmánek et al., 2005; Richardson and Pyšek, 2006), such that a species colonization will not succeed in all recipient areas. Our knowledge about the potential natural establishment of *E. globulus* in different habitats is still scarce. Understanding why some sites are more susceptible to the establishment of a given plant species than others has been a central topic in biological invasions research (Chytrý et al., 2008b). In turn, susceptibility of the receiving community can change in function of competitive and facilitative interactions, nutrient availability, or disturbance levels (e.g. Davis and Pelsor, 2001; Davis et al., 2000; Traveset and Richardson, 2014). Particularly, the role of disturbance have been long recognized as possible important driver of invasion (Elton, 1958). For instance, many studies have observed that disturbance, specifically native vegetation removal, favours exotic plants (e.g. Alston and Richardson, 2006; Davis and Pelsor, 2001; Davis et al., 2000; Higgins and Richardson, 1998; Mitchell et al., 2006), and has been suggested to be a prerequisite for eucalyptus establishment (da Silva et al., 2011; Lorentz and Minogue, 2015; Wevill and Read, 2010).

The number of propagules arriving to the new environment (i.e. propagule pressure) has also been observed as one of the mechanism that strongly influences exotic species colonization success (e.g. Catford et al., 2011, 2009; Krivánek et al., 2006; Lockwood et al., 2009; Pyšek et al., 2009; Rouget and Richardson, 2003) and it is thought to be an especially important factor for eucalyptus population performance (Gordon et al., 2012; Rejmánek and Richardson, 2011). In forestry species, the extension and the intensity of the planted area determine the magnitude of the propagule pressure that could affect the surrounding native vegetation. Eucalyptus propagule pressure can potentially be very high considering the extension of plantations and their prolific seed production (Rejmánek and Richardson, 2011). However, the importance of propagule pressure on establishment success can vary depending on the local conditions (i.e. characteristics of host community). For instance, a low amount of propagules can be sufficient to assure the colonization in a low resistant community (e.g. highly disturbed), whereas in locations with highly resistant communities (with intense competition or harsh abiotic conditions) may be necessary a greater number of propagules (Krivánek et al., 2006; Lockwood et al., 2009; Pyšek et al., 2009; Rouget and Richardson, 2003). Similarly, residence time (i.e. the time since a species was first introduced into a region) can also influence the success of exotic species colonization (Dawson et al., 2009; Pyšek et al., 2009). Considering that data on these aspects is rarely available for most species introduced to a particular location, the existence of detailed records of planting history (and introduction) for forestry species offers an opportunity to access the relative importance of a potential surrogates for propagule pressure and residence time in explaining exotic species natural colonization success.

Recently, Catry et al. (2015) studied the effect of abiotic factors on *E. globulus* natural establishment along roadside transects adjacent to eucalypt plantations in continental Portugal. The study did not consider the characteristics of surveyed plantations and others important site variables, such as vegetation cover and disturbance level. Besides, roadsides adjacent to eucalypts plantations are areas of human-caused disturbance, becoming difficult to generalize to other habitat types. Therefore, we still have a gap of knowledge about the importance of propagule pressure, residence time, and the characteristics of the host community on *E. globulus* natural establishment. In this study, we evaluated how variables related to a region´s climate, propagule pressure (i.e. age, size, productivity and fire recurrence of the plantation) and host site characteristics (i.e. habitat type, soil type, aspect, disturbance level and vegetation cover) are associated with the natural establishment of the exotic planted species, *E. globulus,* into natural habitats. The specific questions are: i) what is the relative importance of site characteristics, propagule pressure, and climate in the natural establishment (i.e. eucalypt occurrence) of eucalyptus from plantations? ii) Once eucalyptus is established, which are the factors associated with the abundance of eucalypts in natural habitats? We assessed *E. globulus* natural colonization in several habitats adjacent to 50 plantations across mainland Portugal (~450 km). A close collaboration with plantation owners offered access to detailed information about *E. globulus* plantation and produced a unique database to estimate the importance of propagule pressure in plant invasions.

METHODS

Study species

In Portugal, *E. globulus* plantations are mostly intensive managed through a coppice system (10–12 year rotations) and their wood is mainly used for pulp production (Soares et al., 2007). *E. globulus* sexual maturity occurs usually at 3-4 years old associated with the change to adult leaves (Jordan et al., 1999) and each fruit (a woody capsule) contains c. 6-25 viable seeds (Hardner and Potts, 1995; Mimura et al., 2009). *E. globulus* seeds are small (but relatively large for eucalypts) and with no specialized dispersal mechanism (Cremer, 1977). Moreover, since *E. globulus* seeds are mainly dispersed within the fruits, capsules are the main diaspores (Calviño-Cancela and Rubido-Bará, 2013). Thus, seed dispersal distances seem to be limited to 10–15 m from plantation edge (Calviño-Cancela and Rubido-Bará, 2013; P. Fernandes et al., 2016). Its fruits are able to withstand fire and be stimulated to release the seeds (i.e. serotionous) (Reyes and Casal, 2001). *E. globulus* seeds show no dormancy and germination is likely to take place within a year after seed release (Calviño-Cancela and Rubido-Bará, 2013).

Study area

We sampled 50 *E. globulus* plantations in a country-wide field survey to document eucalypt establishment (natural regeneration) in the surrounding areas of the managed forest plantations in Portugal (Fig.1). We targeted plantations adjacent to natural habitats (at least one edge in contact with natural habitat) and of a reproductive age. The average age of sampled plantation was 12 years, ranging from 8 to 26 years (Table 1). The average site elevation was 216 m a.s.l (ranging from 14 m 748 m). The climate is Mediterranean: cool rainy winters and hot dry summers. The average of annual mean temperature of the sampled sites was 15ºC. The annual precipitation was 885 mm, ranged to 540 mm from 1438 mm (Table 1). The major soil types found among sampled sites were cambisol, luvisol, podzol and lithosol (Table 1).

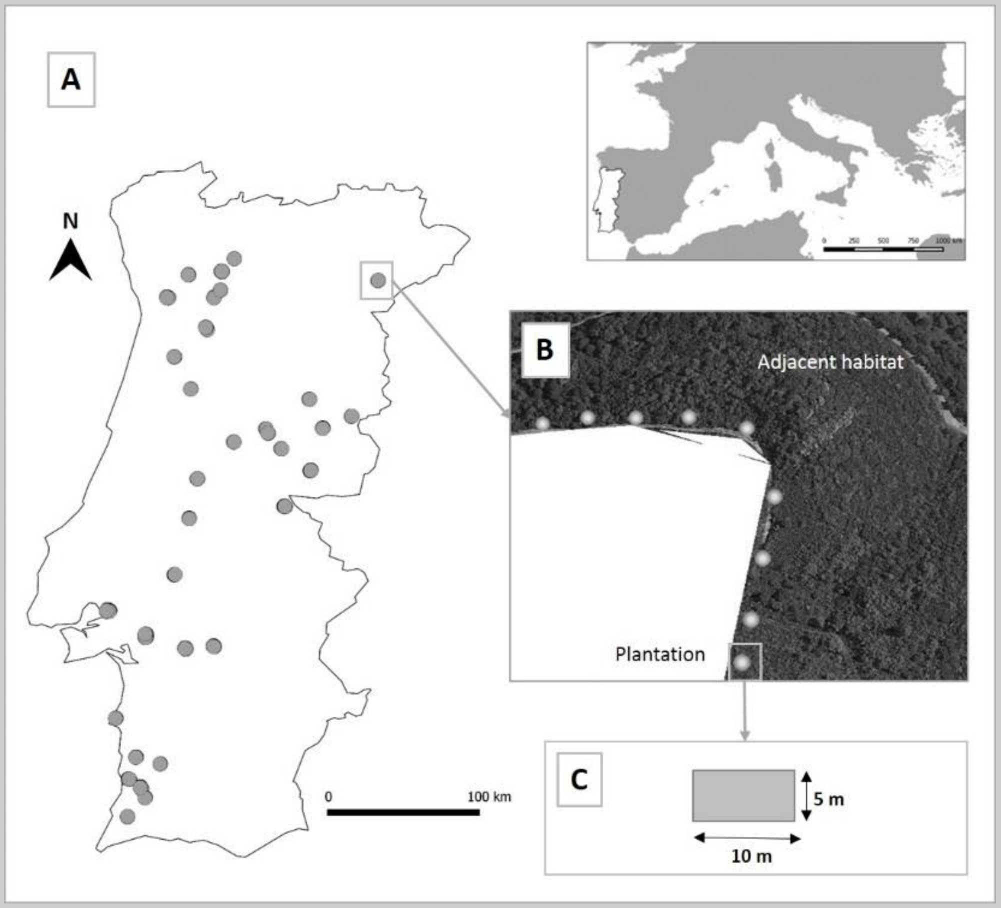


FIGURE 1. A) Location of the 50 studied *Eucalyptus globulus* plantations (in grey dots) in Portugal continental. B) Grey dots adjacent to plantations boundary edge correspond to survey plots. C) Schematic diagram of plots area (10 m x 5 m) which were established perpendicular to plantation edge.

Survey method

The field surveys were conducted between May of 2012 and May of 2014, during the spring and autumn seasons. We perform a series of parallel plots of 50 m^2 (10m x 5m) to quantify the establishment *E. globulus* (i.e. number of plants) in the adjacent habitats of the managed forest plantations of *E. globulus* across Portugal (Fig. 1). *E. globulus* plants counted in this study correspond to all *E. globulus* plants that had been naturally established from planted trees irrespectively of its size. The eucalypt seedlings registered in our study had in most of the cases less than 1.3 m. In total, 1579 plots were surveyed across the 50 plantations of the study area (Table 2). The plots were established with the longer edge perpendicular to the plantations boundary edge. This method was chosen because it allows a high level of detection, being designed to be effective for the detection of rare events (Green and Young, 1993). To calculate the confidence level of the survey (i.e. the probability of sampling eucalypts at least once given a particular value of plots), we applied the following equation (see details in Green and Young (1993)) that gives estimates of how many samples (i.e. plots) must be taken to achieve detection of events (i.e. eucalyptus occurrence):

$$n = -\frac{1}{m} * (\log \beta),$$

were, in this study, n = the number of plots needed to detect eucalypt plants, m = the mean frequency of occurrence of eucalypt, and β = confidence level of the estimate, in the terminology of Green and Young (1993). In practice, it´s very difficult to determine m before the end of the sampling, and therefore must be defined a priori. We adopted m = 0.1 for as one definition of “rare” (Green and Young 1993). Based on this equation we calculated the number of plots needed to achieve a high confidence level (the value of β). Before the survey beginning, we determine a realistic number of 30 plots per site resulting in a confidence level equal to 0.95. The spacing between the plots at each plantation were determined by taking the total length of the plantation boundary and dividing by 30, with the resulting value used as a standard distance between sampling plot. Except in case of very small plantations (when the plantation perimeter was less than 500 m), where the distance between plots was automatically set to approximately 15 m. Areas with very rough terrain (e.g. boulders, ravines, or steep slopes) were not considered in the study. In this sense, the number of plots at each site varied in function upon the size of the sampled plantation and the accessibility of adjacent habitat. The total number of plots ranged from 8 to 58 plots per plantation. Consequently the values of β varied from site to site (β mean = 0.92 ±0.09). The geographic coordinates of each plot centre were registered using a hand-held GPS (Garmin, GPSMAP 76CSx).

Explanatory variables

We characterized each sampled plot with a broad range of variables as potential surrogates for biotic and abiotic conditions that can determine the susceptibility of the site for eucalypts establishment (Table 1). In each plot we estimated the percent cover of herbs and shrubs, percent tree canopy cover, and percent of non-bare ground cover (i.e. the cover of plants and rocks = the opposite of percent of bare soil). We used a quadrat of $1m^2$ to help us to calculate the percent cover of vegetation in each plot. Tree canopy cover of each forested plot was estimated as the sum of the projected crown area inside the plot limits. Within each of sampling plot we also registered the disturbance level. We visually classified the disturbance level using a categorical range of three levels – undisturbed or low disturbed, medium and highly disturbed. Areas with intact vegetation structure and low animal trampling were deemed areas of low disturbance or undisturbed, plots with sporadic to moderate debris or moderate animal/human trampling were considered areas of medium disturbance while patches with high human trampling and accumulation of debris, dumping, logging and high soil erosion were deemed areas as highly disturbed. We registered aspect for each plot (correspond to plantation edge orientation). To facilitate interpretation and decrease the models complexity, we transformed S, SE and SW aspects into an unfavourable group where radiation and evapotranspiration are expected to be higher and the remaining aspects were classified as favourable (Beers et al., 1966; Kutiel and Lavee, 1999). Plots were established in areas with moderate topography (i.e. 5% in average) in order to minimize the effect of slope in the relation of eucalypt density among sites. Slope and aspect could interact to change radiation levels. However, as plots were established in sites with moderate topography, we regarded aspect as a sufficient indicator of light penetration (González-Moreno et al., 2013b). The habitat type where we established each sampling plot was registered in the field: a) Forest, mainly small woods with *Quercus robur*, *Quercus pyrenaica* and *Quercus faginea*); b) shrubland, mediterranean garrigue composed by a mix of short and medium shrubs (until 2 m of height) as rockrose (*Cistus spp.*) and others Cistaceae, heather (genus Erica and Calluna), gorse (Ulex spp), broom (Cytisus spp) and many aromatic species (e.g. *Lavandula stoechas*, *Rosmarinus officinalis*, *Thymus vulgaris*); and c) grassland occupied by rich annual herbaceous species. Shrublands were the dominant habitat type surrounding forest plantations, as a result of agricultural land abandonment. Soil type in each plot was extracted from the Portuguese Atlas of Environment (APA 2013; scale 1:1,100,000).

We characterized the plantations based on their age, residence time, area, productivity and fire history as a proxy to the capacity of propagule production and the dispersal ability (i.e. propagule pressure) (Table 1). The age of plantation corresponds to time (years) from the trees establishment (if it’s the first plantation establishment) or from the last time that trees were cut, to the date of survey. The residence time was calculated based on the number of plantation rotations and cycles (the first cycle corresponds to the first plantation establishment, including 2-4 rotations, and the next cycles correspond to replanting after 2-4 rotations). The productivity of plantation was categorized in three levels – low, medium and high – based on climate and soil suitability for *E. globulus* growth (Soares et al., 2007; Tomé et al., 2001). The fire history was quantified as the presence or absence of big fire events in the last decade (mostly corresponding to 2003 and 2005). All plantation variables were obtained from the plantation owners.

Finally, to account for climate influence, we selected seven climate variables that seemed ecologically most relevant for establishment success (i.e. plant growth and survival; Ibáñez et al. 2009) (Table 1). We selected mean annual temperature, mean minimum temperature of the coldest month, mean maximum temperature of the warmest month (these two last variables inform us about the tolerance limits of *E. globulus* with respect to temperature), temperature seasonality and precipitation seasonality (i.e. coefficient of variation in temperature/precipitation along the year), annual precipitation and mean precipitation of warmest quarter (which provides a proxy for dryness during summer months). Climate data are averages for 1950-2000 downloaded from the WorldClim data base (Hijmans et al., 2005). Each of the plot centre location was intersected with climate data at a 30 arcsec resolution (~1 km).

Data analysis

Considering the high number of zeros in the data, we used separate models to analyse occurrence (i.e. presence/absence of eucalypts) and density data (i.e. density models only used data from occupied plots). This approach solves the overdispersion problem and the potential bias in parameter estimation when modelling zero-inflated data. Furthermore, the results of both models reflect the different stages of plant colonization and they are interpreted as two coupled process (Catford et al., 2011; González-Moreno et al., 2014; Ibáñez et al., 2009). Since there is no reason to expect factors to be of equal importance for both presence and density stages, this two-stage modelling process ensured that factors that contribute to high density could be identified regardless of whether sites are presently occupied by eucalypts (Catford et al., 2011). We analyse the association of occurrence of eucalypts (i.e. presence/absence data in 1579 plots) and subsequent density (i.e. number of plants) with site characteristics, propagule pressure and climate (Table 1) by multimodel inference (Burnham and Anderson, 2002) and variance partitioning techniques (Mood, 1969) based on generalized linear mixed models (GLMM) with the identification of plantation as a random effect to account for pseudoreplication.

TABLE 1. Variables used as a predictors' *E. globulus* occurrence and density in habitats surrounding plantations.

Variable	Description	Mean (SD)	Range
Site characteristics			
Habitat type [a]	Three habitats types: forest, shrublands and grasslands.	-	-
Soil type [a]	Major soil type (5 *cat.*): cambisol, lithosol, luvisol, podzol and others.	-	-
Aspect [a]	Aspect (2 *cat.*): unfavourable (SE, S and SW aspects) and favourable (remaining aspects)	-	-
Disturbance [a]	Categorical range of 3 levels: 1) undisturbed –low; 2) medium; 3) high.	-	-
Shrubs cover (%)		51.24 (27.88)	0 - 100
Herbs cover (%)		16.27 (25.67)	0 - 100
Canopy cover (%) [a]		12.98 (23.76)	0 - 90
Non-bare ground cover (%) [a]		74.34 (18.36)	10 - 100
Propagule pressure			
Plantation age (years) [a]		11.57 (2.62)	8 – 26
Residence time (years) [a]		29.63 (14.02)	15 – 68
Plantation area (ha) [a]		9.29 (10.36)	0.45 - 45.18
Productivity region [a]	Categorical range of 3 levels: 1) Low; 2) Medium; 3) High	-	-
Fire occurrence [a]	2 cat.: 1 (yes), or 0 (no)	0.36 (0.48)	0 - 1
Climate [b]			
Mean annual temperature (ºC)		15.23 (1.44)	11.6 – 17
Temperature Seasonality [a]		4734.11 (582)	3816 - 5914
Mean minimum temperature (ºC January)		5.45 (1.95)	1.8 – 8.4
Mean maximum temperature (ºC July)		28.54 (1.75)	25.1 – 32
Annual Precipitation (mm) [a]		884.83 (273.7)	540– 1438
Precipitation Seasonality		57.28 (5.15)	49 - 67
Summer precipitation (mm)		56.67 (28.43)	21 - 108

cat.: categories

[a] Variables included in the final analysis to avoid collinearity among continuous variables.

[b] Data source: WorldClim (Hijmans et al. 2005)

Because of the very low number of plots with eucalypts presence in grassland (n = 3) and forest (n = 2) habitats, habitat type factor was no considered in the density model. This approach facilitated model convergence and results were similar if data is restricted to the main habitat type (i.e. shrublands; See Table A.1). The final density data set has information from 120 plots. Occurrence was modelled by setting a binomial distribution for the response variable and a logit link (presence model) (Quinn and Keough, 2002). The density was modelled using a negative binomial distribution, which is typically used for counted data when overdispersion occurs (Gelman and Hill 2007). Preliminary analysis using Moran´s Index, indicated no relevant spatial autocorrelation in the model´s residuals (I<0.4) (Fig. A.1).

Prior to modelling we checked the explanatory variables for collinearity using pair-wise Pearson´s correlation test, excluding correlation values higher than 0.6 and then selected ones with best explanatory power (Table A.2 and A.3). With regard to site variables, shrubs cover and herbs cover were correlated with non-bare ground cover. For final model we used the percent of non-bare ground cover and canopy cover site variables. We kept all propagule pressure variables. Climate variables were highly correlated and thus we selected only two non-collinear variables: temperature seasonality and annual precipitation.

Multimodel inference, the model selection method used in this study, provides a framework to ‘evaluate the relative strength of evidence in data for multiple hypotheses represented as models’ (Hobbs and Hilborn, 2006). This method provides two approaches to find the best models to use: model selection and model averaging. Thus, multimodel inference method allowed us to identify the best possible models and rank all explanatory variables according to their importance on eucalyptus occurrence and density (Burnham and Anderson, 2002). We performed multimodel inference based on all-subsets selection of GLMMs using Akaike´s information criterion corrected for a large number of predictors (AICc) (Burnham and Anderson, 2002; González-Moreno et al., 2013a). First, for each group of predictors (i.e. site, propagule pressure and climate) we selected the best model (smallest AICc). Next, the same procedure was performed combining the best variables of each group to establish the set of best candidate models. All models within four AICc units from the best model (delta < 4) were considered as the set of best models given the selected predictors. This threshold is within the limits adopted in other studies (González-Moreno et al., 2013a; Grueber et al., 2011). Within the best models subset we calculated the Akaike weight of evidence (w_i) for each candidate model to rank the predictors in order of importance in their relation to response variables. The w_i of each predictor was estimated as the sum of the model AICc weights across all models in which the selected predictor appeared. This means that the predictor with highest w_i (i.e. closest to one) gets the highest relative importance (compared to the rest of predictors) to explain the response variables within the given data (Burnham and Anderson, 2002). In addition, multimodel inference was used to estimate regression coefficients and their confidence intervals (with adjusted standard error) within the best models subset (delta <4). Coefficient of a given selected predictor was calculated as the sum of the predictor´s coefficient multiplied by the weight of evidence, across all possible models where the predictor is present (Burnham and Anderson, 2002).

Variance partitioning techniques indicate the variability explained by the single and shared groups of variables (Mood, 1969). We set up the best GLMMs selected in the previous approach for each dependent variable and incorporating only site variables, only propagule pressure variables, only climate variables and the combination of these three groups of variables. Considering that the deviance is a good measure to variability explained by a model, we used the deviance explained by each model to identify the pure and mixed effects by simple equation systems (Carrete et al., 2007; González-Moreno et al., 2013a). Following the same approach, we also partitioned the deviance of eucalyptus presence and density accounted by site characteristics within three best site factors explaining each dependent variable. We also performed the variance partitioning analysis including only the two of the most important non-collinear variables of each group, in order to compare the differences between the results with all variables and with the same number of variables by group. The results did not show significant differences between these two approaches (Fig. A.2). We only present the results for all variables.

All statistical analyses were performed with the R-CRAN software (R Development Core Team 2009). We used the package MuMIn for some procedures of the multimodel inference method and the package VEGAN as the base code for deviance-partition.

RESULTS

Differences in E. globulus occurrence probability among habitat types

Eucalypts presence was recorded in 120 plots, representing 8% of the total plots performed (Table 2). The survey resulted in a detection of 480 eucalypts (Table 2), ranging between 0 and 20 individuals per plot. Natural regeneration of eucalyptus occurred mainly in shrubland with eucalyptus presence in 12 % (115 plots) of the plots sampled in this habitat and counting a total of 468 plants (Table 2). Considering the best occurrence model (see Table A.4), forest and grassland were the most resistant habitat to eucalyptus establishment (Table 2).

TABLE 2. A summary of the habitat types at each of plot sampled from all sites surveyed, and the description of eucalypts among the habitat types. *Letters* indicate significant differences (p>0.05) among habitat types according to post-hoc test corrected for multiple hypothesis based on the best occurrence model selected by AICc criteria.

Habitat type	Number of plots	Number of eucalypts	Number of plots with eucalypts	Proportion of plots with eucalypts
Forest	409	4	2	0.005^{a}
Grassland	215	8	3	0.014^{b}
Shrubland	955	468	115	0.120^{c}
Total	1579	480	120	0.076

Partitioning the influence of climate, propagule pressure and site characteristics on eucalyptus natural regeneration

The variability of both occurrence and density of eucalyptus was explained mainly by site variables (Fig. 2). The site variables had very high single effect on eucalypts occurrence (57%; Fig.2A-i). In contrast, climate and propagule pressure had very low single effect and its explanatory power was shared mainly with site variables (Fig.2A-i). Considering site variables separately (the best three site variables), the deviance partitioning-analyses revealed that the percent of non-bare ground cover of the areas surrounding plantations explained alone most of the deviance of eucalyptus occurrence (47%; Fig.2A-ii). The explanatory power of habitat type was partially shared with percent of non-bare ground cover (3%) and account 7% of single effect. The effect of disturbance on eucalypts occurrence was totally shared with percent of non-bare ground cover (Fig.2A-ii).

The deviance partitioning indicates that a combined effect of the propagule pressure and site variables accounted for the largest fraction (22%) of the variability of the eucalyptus density, followed by the combined effect of climate and site variables (18%). The largest independent effect is accounted for site variables (17%) and the independent effect of the others two groups was practically inexistent (Fig. 2B-i). Considering site variables, the variability of eucalyptus density was explained mainly by the single effect of disturbance (17%), followed by their combined effect with non-bare ground cover (13%). Percent of non-bare ground cover had a low single effect (8%) on eucalyptus density. The total amount of aspect effect was shared with disturbance and non-bare ground cover (Fig.2B-ii).

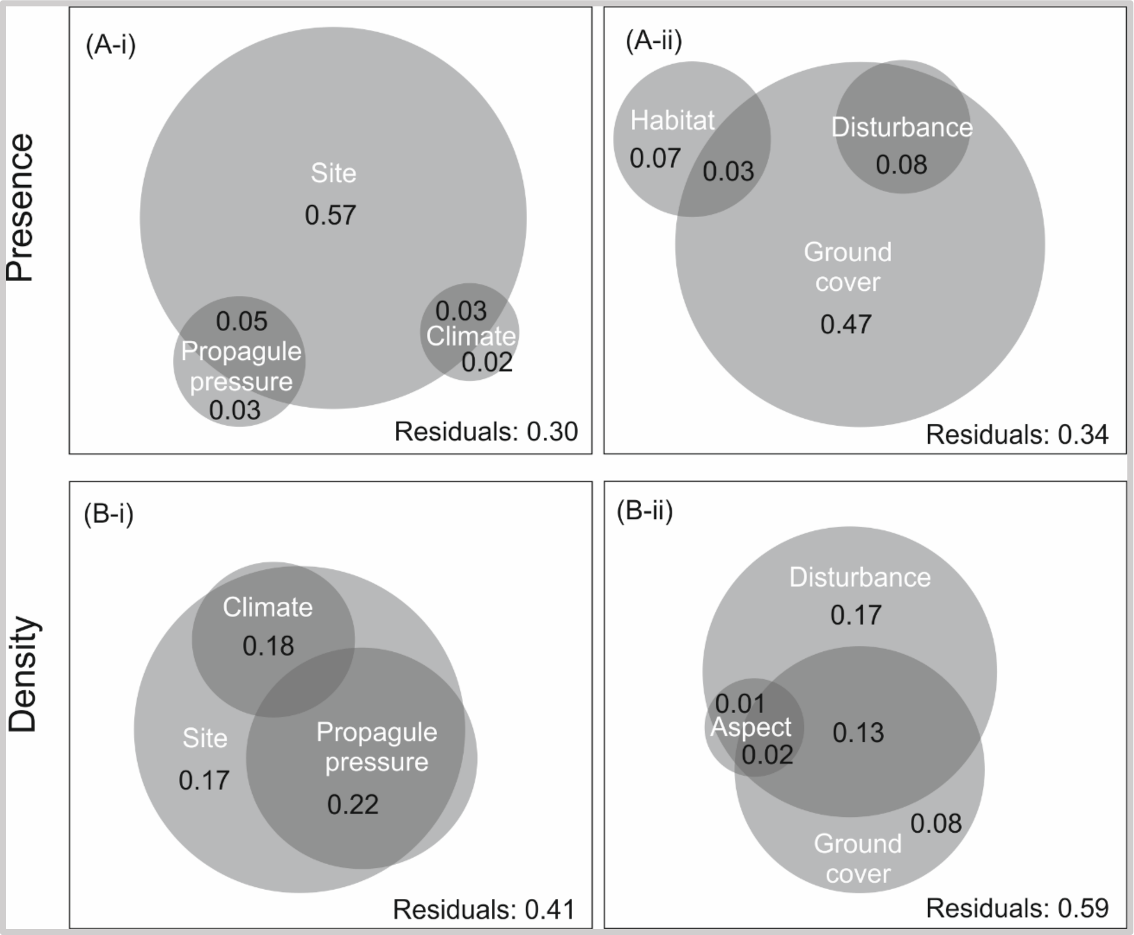


FIGURE 2. Deviance partitioning of *E. globulus* presence (A-i) and density (B-i) using generalized linear mixed models among climate, propagule pressure and site characteristics predictors, and deviance partitioning of *E. globulus* presence (A-ii) and density (B-ii) using generalized linear mixed models among the three best site factors explaining each dependent variables. Each circle corresponds to a group of variables. Numbers within circles are the proportion of deviance explained by each set of predictors alone (non-overlapped part of circles) or shared. Residuals indicate the deviance non-explained by the models.

The association of climate, propagule pressure and site characteristics with eucalypts natural regeneration

The variables best explaining eucalyptus occurrence and density were different. In the case of density data analysis, no climate and propagule pressure variables were selected (Table 3; Table A.5). These results indicate that once eucalypts have high probability of establishment then, the only aspect that matters to the eucalypts success is the site conditions.

The major correlates (i.e. higher weight of evidence) for eucalyptus presence were site factors: habitat type and non-bare ground cover. After site factors, productivity region (the best propagule pressure predictor explaining eucalyptus occurrence) was the next in importance to model fit, followed by annual precipitation (the best climate factor) (Table 3). Specifically, we found higher probability of presence of eucalyptus at shrubland habitat and lower at forest habitat (Table 2). The probability of eucalyptus occurrence was lower at low productivity regions (Fig. 3). Non-bare ground cover showed a significant negative association with eucalyptus occurrence. Finally, annual precipitation had a weak positive relation with eucalyptus occurrence (Table 3).

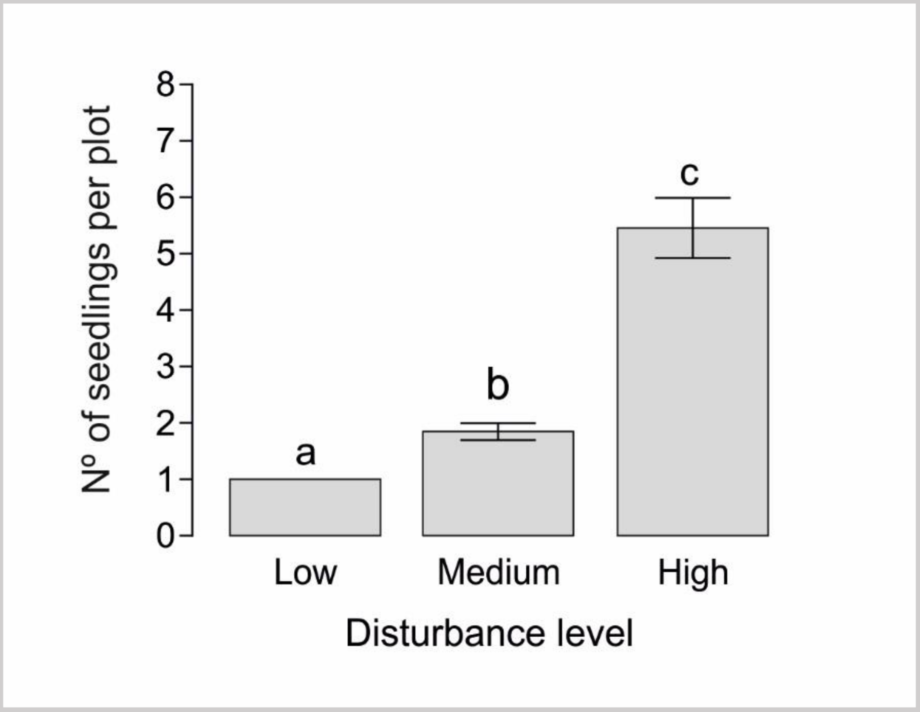


FIGURE 3. Mean (±SE) eucalypts density (nº of eucalypts/plot) for low (N = 2), medium (N = 46) and, high (N = 72) disturbance levels. *Letters* indicate significant differences ($p > 0.05$) between disturbance levels according to pos hoc test corrected for multiple hypothesis based on the best density model selected by AICc criteria.

The variables best explaining eucalyptus density were disturbance and non-bare ground cover (Table 3). Considering the best density model (Table A.5), eucalyptus density had a significant negative association with non-bare ground cover and was significantly higher in the highly disturbed plots (Fig.3). Aspect also had some importance explaining eucalyptus density, with slightly higher density (no significant) in northern aspect (N, NE and NW) (Table 3).

TABLE 3. Multimodal inference results: weight of evidence, averaged coefficient estimates, adjusted standard error and confidence intervals (95% CI) of climate, plantation and site predictors for *E. globulus* occurrence in surrounding plantations habitats. Type of predictors: S = site characteristics; PP = propagule pressure; C = climate. Habitat type was not considered in the Density model (see Methods).

Variable	Type	W	β Averaged		Adjusted SE	Lower CI	Upper CI
Presence							
Intercept			-12.191	***	1.847	-15.796	- 8.550
Habitat type	S	1.00					
Non-bare ground %	S	1.00	- 3.879	***	0.433	- 4.726	- 3.029
Productivity region	PP	0.84					
Annual precipitation	C	0.46	0.372		0.615	- 0.566	2.173
Density							
Intercept			1.173	***	0.236	0.646	1.662
Disturbance	S	1.00					
Non-bare ground %	S	0.86	- 0.162	*	0.073	- 0.304	- 0.020
Favourable aspect	S	0.69	0.374		0.207	- 0.032	0.781

Predictors of each model are sorted by importance according to the weight of evidence. Regression coefficients for the categorical variables habitat, productivity region and disturbance are not shown .Significance of averaged coefficients: p<0.001: ***; p<0.05: *. See table 1 for a complete description of variables.

DISCUSSION

Conceptual models to explain exotic plants establishment have pointed to the combination of having enough propagule pressure and a suitable environment, biotic and abiotic (Catford et al., 2009; Chytrý et al., 2008a). In this study, climate was used to estimate abiotic suitability (Walther et al., 2009), plantation characteristics as a proxy for propagule pressure (Pyšek et al., 2009), and site characteristics (including habitat type, vegetation cover and disturbance) mainly representing characteristics of the host community (biotic and abiotic suitability) (Rejmánek et al., 2005). Among these variables, we found site characteristics the most important group of variables influencing *E. globulus* establishment (occurrence and density) outside planted areas in comparison to climate and propagule pressure. This pattern reflects that most of the variability in Eucalyptus establishment occurs at small spatial scale (site scale; <1 km) (Milbau et al., 2009a). Factors affecting species distribution most likely do so in a hierarchical manner, with different ecological factors affecting plant establishment success more strongly at different spatial scales (Milbau et al., 2009). For example, Pearson et al. (2004) studied the impact of climate and land cover changes on species distributions in Britain and discovered a dominant effect of climate measured at the European scale over land-cover data at the British scale. In accordance with this, climate and propagule pressure might control variability in *E. globulus* establishment among plantations (regional scale: 2,000-200 km), while site characteristics influence *E. globulus* establishment within plantations' area (site scale: 10 – 1,000 m). At coarser scales such as continental, climate would probably have a more important role shaping *E. globulus* natural establishment (Catry et al., 2015; Larcombe et al., 2013). Despite the scale of our study, we still found a relative high importance of annual precipitation as best climate predictor. We observed that *E. globulus* was more likely to be present in areas with high annual precipitation. Previous studies pointed at the same direction (Águas et al., 2014; Catry et al., 2015; Larcombe et al., 2013). Catry et al. (2015) found that eucalyptus density were lowest below c. 600 – 700 mm of annual precipitation and then sharply increased up to around 1500 mm. They also found that eucalyptus density reached the lowest values above 2400 mm. The maximum annual precipitation sampled in our study was 1438 mm and, 885 mm in average, which seems to be the optimal for eucalyptus establishment.

The identity of the habitat and the percentage of non-bare ground cover were the most important factors explaining eucalypts occurrence. Previous studies have also found habitat type to be the foremost factor explaining differences in plant invasions (Chytrý et al., 2008a; Gassó et al., 2012). Moreover, studies have pointed out that alien species traits related to successful establishment are habitat dependent (González-Moreno et al., 2013b; Lake and Leishman, 2004; Lloret et al., 2005). The probability of eucalyptus occurrence was higher in shrubland habitat than in forest and grassland. Shrubs can have a nurse effect for eucalyptus seedlings protecting them from harsh conditions (Gómez-Aparicio et al., 2008). Shrublands might also provide higher light availability for later stages in the development of seedlings. *E. globulus* have been classified as shade-intolerant species, requiring a high light regime to develop (Correia et al., 1989). Native forests dominated by pedunculated oak, as other broad leaved trees, have low light irradiance regimes at ground level compared to open vegetation of shrubland (Barbier et al., 2008). Therefore, the lowest probability of eucalyptus occurrence found in forest patches is likely to be caused by a negative shading effect. Likewise, Calviño-Cancela and Rubido-Bará (2013) showed lower *E. globulus* seedling emergence under native forest patches (pedunculated oak) than in the shrubland. This species is also reported as highly sensitive to drought, particularly at the seedling stage (e.g. Stoneman, 1994). Eucalypts produce very large quantities of seeds but very small with no obvious endosperm. Thus, the newly emerged seedlings (sustained by cotyledon photosynthesis) need that their roots penetrate into suitable wet substrate very quickly to survive (Rejmánek and Richardson, 2011). Grasslands normally show high seasonal fluctuation in water soil and nutrients availability (Rundel et al., 2014). These factors could affect negatively eucalyptus seedling survival in this particular open habitats (Rejmánek and Richardson, 2011). High mortality during the initial phase of seedling growth due to unsuitable environmental conditions or competition with other plants have been also mentioned by others studies as one of the most important factor that limits eucalyptus colonization (Callaham et al., 2013; da Silva et al., 2011; Rejmánek and Richardson, 2011).

The survival of eucalyptus seedlings is generally low in dense vegetation sites (Rejmánek and Richardson, 2011). This is in agreement with the significant negative association found in our study between non-bare ground cover percentage (mainly determined by understory vegetation cover) and the probability of eucalypts occurrence and density. Non-bare ground cover alone explained 47% of eucalypts occurrence variability. Therefore, the higher probability of eucalyptus establishment is mainly related to the horizontal structure of the community. Vegetation structure could be the responsible of the low establishment rate found in comparison to other studies. In Catry et al. (2015), eucalypts presence was found in 60% of the roadside transects adjacent to eucalypt plantations. On the contrary, we only found establishment in 8% of the total plots. Roadsides usually have high percentage of exposed soil without vegetation. This characteristic seems to increase the suitability of community to eucalypts establishment, increasing the window of opportunity for eucalypt seeds to reach suitable sites.

The identity of the productivity region was, after site variables, the most important factor explaining eucalypts occurrence and the best propagule pressure predictor. This factor is based on climate and soil suitability for *E. globulus* growth (Soares et al., 2007; Tomé et al., 2001). Thus, as climate factors, productivity region can be also considered as a proxy to abiotic suitability to eucalyptus growth at regional scale. The probability of eucalyptus occurrence was lower at low productivity regions although no significant effect was found. The partition analyses revealed that propagule pressure had a low explanatory strength and shared its explained deviance with site characteristics. The intensive management of these plantations could explain these results. Industrial *E. globulus* plantations are grown with very short rotations (rotation time in Portugal is 10-12 years) and with a continuous management. Thus, the low age of *E. globulus* plantations might contribute to diminish their ability to develop a significant propagule pool. The importance of plantation characteristics might increase in abandoned plantations (not considered in this study), as the propagule pressure builds with time. Thus, future research in abandoned eucalypt plantations is needed to better understand the role of propagule pressure and residence time in *E. globulus* colonization.

Eucalyptus occurrence and density were modulated by different variables. In contrast to occurrence, best models for density data only included site characteristics. Additionally, the partition analyses showed that the total amount of propagule pressure and climate effects was completely shared with site factors. Thus, the independent effect of these two groups of factors was practically inexistent. These results indicate that once eucalypts have high probability of establishment then, the only aspect that matters to the eucalyptus success is the characteristics of the host community. This result is in accordance with the idea proposed by Milbau et al. (2009), suggesting small-scale factors being better predictors at finer scales if conditions at higher levels are satisfied. The disturbance level was the most important factor explaining eucalyptus density. Disturbance, specifically native vegetation removal, is an important driver of alien species expansion (e.g. Alston and Richardson, 2006; Davis and Pelsor, 2001; Davis et al., 2000; Elton, 1958; Higgins and Richardson, 1998; Mitchell et al., 2006). Our results point at the same dirrection, with an increasing in eucalyts density with increasing levels of disturbance. This result agrees with previous findings by Lorentz and Minogue (2015) and Silva et al. (2011), where litter and plant removal improved the survival of eucalypts seedlings. The eucalypts density found in all high disturbed plots close to plantations (mean ± SD = 293 ± 673 plants ha^{-1}) is quite similar to the mean density calculated by Catry et al. (2015) in roadside transects adjacent to eucalypt plantations (277 plants ha^{-1}), in Portugal. In spite of the different survey methods, both studies have surveys adjacent to plantations and roadsides can be considered a high disturbed environment with high percentage of bare ground. However, the observed average eucalypts density in both studies is very low compared to well-known invasive forestry species (e.g. pines, Richardson et al., 1994) and also with native maritime pine planted in Portugal (892 plants ha^{-1}) (P. Fernandes et al., 2016). Aspect was also one of the site characteristics with effect on eucalyptus density. Higher eucalypt density was found in aspects considered favorable (N, NE and NW). Thus, the expected higher radiation and evapotranspiration under the S, SE and, NW aspects (Beers et al., 1966; Kutiel and Lavee, 1999) limited eucalypts establishment. However, the importance of this variable was considerably lower when compared to ground cover and disturbance level factors.

CONCLUSIONS

In the present study we showed that eucalyptus establishment is limited in the natural habitats adjacent to managed plantations, compared with values recorded on roadsides (Catry et al., 2015). This was particularity true when the habitat type surrounding the sampled plantations was forest or grassland. The percentage of bare ground cover had a significant effect on eucalyptus establishment. From our findings, we can suggest that the increase of bare ground percentage increases the susceptibility to eucalyptus establishment. Indeed, our results suggest that the control of the eventual disturbance in habitats adjacent to plantations during the management activities would also greatly reduce the establishment success of *E. globulus*. In summary, our study suggests that eucalypts establishment is highly limited by biotic interactions (i.e. competition with other plants) and low light and water availability**.** Nowadays, the expansion of trees into habitats outside plantations is a concern for managers and conservationists. These small-scale factors are essential to precise predictions at more local scales. Other higher scale factors, namely climate and productivity region, had lower explanatory strength on eucalyptus establishment probability. As a cultivated tree, *E. globulus* current distribution in Portugal is mainly a result of human activity, targeting environmental conditions highly suitable for the species. Thus, monitoring efforts should focus on the site factors determining the colonization (i.e. ground cover, habitat type and disturbance). Industrial *E. globulus* plantations are grown with very short rotations (rotation time in Portugal is10-12 years) and with a regular basis management, so there are likely to be multiple opportunities for detection and control over the life of a plantation. Take into account our results, we also suggest the establishment of "sentinel sites" using remote sensing tools (e.g. Google Earth) as described by Visser et al. (2014) or in-situ visits during conventional management. This monitoring could, provide an early-warning system for prevention management and over time, it will produce very valuable data contributing to our understanding of eucalypts colonization dynamics.

ACKNOWLEDGEMNTS

This research was funded by Fundação para a Ciência e a Tecnologia (FCT) and the Navigator Company in the frame of Patrícia Fernandes PhD scholarship from FCT (SFRH/BDE/51709/2011). We would like to thank the Navigator Company for providing access to plantations, maps and data. Namely, we would like to thank: André Carvalho, Eduardo Mendes, Luís Alarico, João Bandeira, Américo Campos, Francisco José Inácio, Isidro Costa, João Pires, Joaquim Fonseca, Jorge Lourenço, José Carlos Pereira, Leonel Almeida and Ricardo Mendes. We also express our gratitude to Sara Coelho, Elsa Gonçalves, Andreia Anjos and Márcia Vieira for the help collecting data during field surveys.

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APPENDIX

TABLE A1. Multimodal inference results: weight of evidence, averaged coefficient estimates, adjusted standard error and confidence intervals (95% CI) of climate, plantation and site predictors for *E. globulus* density in shrubland habitat (n = 115).

Variable	Type	W	β Averaged	Adjusted SE	Lower CI	Upper CI
Intercept			1.163 ***	0.256	0.646	1.662
Disturbance	S	1.00				
Non-bare ground %	S	1.00	- 0.184 *	0.061	- 0.328	- 0.041
Favourable aspect	S	0.59	0.228	0.257	- 0.060	0.835

Predictors of each model are sorted by importance according to the weight of evidence. Regression coefficients for the categorical variable disturbance are not shown. Significance of averaged coefficients: $p<0.001$: ***; $p<0.05$: *.

TABLE A.2. Pearson´s correlation coefficients among the predictors (only continuous variables) used to model the occurrence of *E. globulus* in habitats surrounding plantations (n = 1579). Significance: p<0.001 ***; p<0.01 **; p<0.05 *.

	Area (ha)	Age (years)	Time of introduction	Temperature seasonality	Annual Precipitation	Non-bare ground cover
Age	- 0.270 ***					
Time of introduction	- 0.334 ***	0.433 ***				
Temperature seasonality	- 0.367 ***	0.334 ***	0.211 ***			
Annual Precipitation	- 0.526 ***	0.285 ***	0.460 ***	0.276 ***		
Non-bare ground cover	0.094 ***	- 0.018	- 0.051 **	- 0.005	-0.144 ***	
Canopy cover	- 0.005	0.065 ***	- 0.037 *	0.029	-0.084 ***	-0.095 ***

TABLE A3. Pearson´s correlation coefficients among the predictors (only continuous variables) used to model the density of *E. globulus* in habitats surrounding plantations (n = 120). Significance: p<0.001 ***.

	Area (ha)	Age (years)	Time of introduction	Temperature seasonality	Annual Precipitation	Non-bare ground cover
Age	- 0.330 ***					
Time of introduction	- 0.136	-0.366 ***				
Temperature seasonality	- 0.501 ***	0.468 ***	-0.009			
Annual Precipitation	- 0.525 ***	0.331 ***	-0.348 ***	0.483 ***		
Non-bare ground cover	0.149	0.034	0.072	0.045	-0.004	
Canopy cover	- 0.009	0.102	- 0.083	0.021	-0.041	0.012

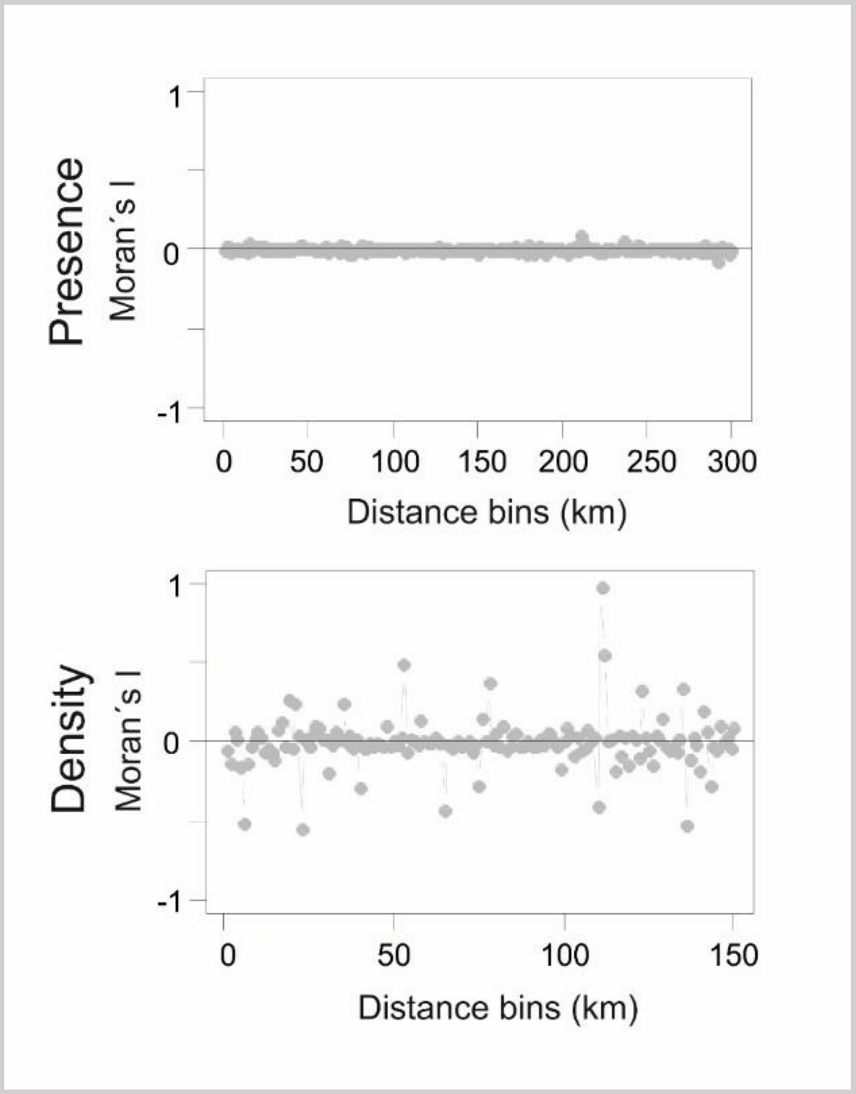


FIGURE A1. Moran´s Index calculated in 1 km bins for the models explaining the presence and density of *Eucalyptus globulus* natural regeneration.

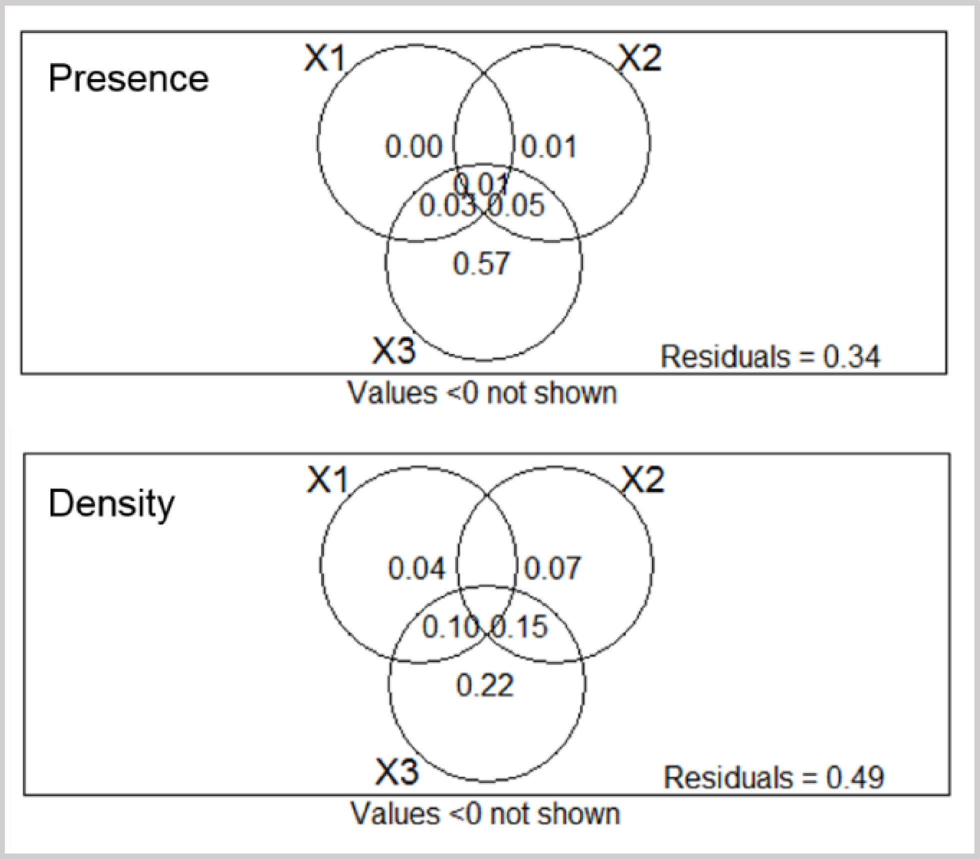


FIGURE A2. Deviance partitioning of *E. globulus* presence and density using generalized linear mixed models among climate (X1), propagule pressure (X2) and site characteristics (X3) predictors. Each group of predictor included only the two of the most important non-collinear variables explaining each dependent variables. Each circle corresponds to a group of variables. Numbers within circles are the proportion of deviance explained by each set of predictors alone (non- overlapped part of circles) or shared. Residuals indicate the deviance non-explained by the models.

TABLE A4. Selection of best models considering AICc criteria (Delta<4: all models within 4 AICc units from the best model) for eucalyptus occurrence considering climate, plantation and site variables. In bold the best model considering climate.

Model	df	logLik	AICc	Delta	Weight
3+4+5	7	-120.79	255.66	0.00	0.26
1+3+4+5	**8**	**-119.89**	**255.86**	**0.21**	**0.24**
2+3+4+5	8	-119.96	256.00	0.35	0.22
1+2+3+4+5	9	-119.62	257.36	1.70	0.11
1+3+5	6	-123.18	258.42	2.76	0.07
3+5	5	-124.39	258.81	3.15	0.05
1+2+3+5	7	-122.63	259.34	3.68	0.04

1: Annual precipitation; 2: Temperature seasonality; 3: Habitat type; 4: Productivity; 5: Understory cover.

TABLE A5. Selection of best models considereing AICc criteria (Delta<4: all models within 4 AICc units from the best model) for eucalyptus density considering climate, plantation and site variables.

Model	df	logLik	AICc	Delta	Weight
1+2+3	**7**	**-250.37**	**515.75**	**0.00**	**0.54**
2+3	6	-252.03	516.81	1.06	0.32
1+2	6	-252.84	518.43	2.68	0.14

1: Aspect; 2: Disturbance; 3: Understory cover.

CHAPTER 4

COMBINED EFFECTS OF CLIMATE, HABITAT, AND DISTURBANCE ON SEEDLING RECRUITMENT AND SURVIVAL OF *PINUS PINASTER* AND *EUCALYPTUS GLOBULUS*

Fernandes, P., Máguas, C. & Correia, O. Combined effects of climate, habitat and disturbance on seedling recruitment and survival of *Pinus pinaster* and *Eucalyptus globulus*. *In review in Plant Ecology*.

ABSTRACT

Nowadays, the natural expansion of forestry trees into habitats outside plantations is a concern for managers and conservationists. This study aims to evaluate the establishment capacity of *P. pinaster* and *E. globulus* in an array of ecological conditions. We measured establishment success in terms of seedling emergence and seedling survival capacity. We investigated the effects of climate, habitat and disturbance on seedling emergence and survival using a seed addition experiment. Both species recruitment were limited in the xeric site and under forest habitats. However, these species were influenced by interactive effects of site and habitat. In the mesic site the negative shading effect by forest canopy on these species establishment was stronger than the effect of water availability. On the other hand, under xeric conditions the effect of drought were stronger than the effect of light. *P. pinaster* was not significantly affected by disturbance. In contrast, we observed a positive effect on *E. globulus* establishment by removing vegetation layer, although only in the shrubland habitat at mesic site. The survival percentage of *E. globulus* was significantly lower than *P. pinaster* (0.27% vs 7.96%). The effects of drought, light availability and disturbance on these species establishment were context-dependent. These results may help to assess the impact of environmental conditions on these species establishment, and can help us to explain future shifts in these species distribution and natural establishment outside the planted areas.

INTRODUCTION

Forest plantations are one of the main sources of plant invaders worldwide (Richardson, 1998). Nowadays, the natural expansion of forestry trees into habitats outside plantations is a concern for managers and conservationists, and a relevant issue for ecology research (e.g. Dodet and Collet, 2012; Richardson and Rejmánek, 2011; Richardson, 1998; Richardson et al., 2013). Among the most planted species worldwide, we can find Eucalyptus and Pinus species (Richardson and Rejmánek, 2011). The use of these species in forest plantations is primarily related to their wide ranges of ecological requirements, to their rapid growth and high productivity (Richardson and Rejmánek, 2011). However, these characteristics and their widespread use in forestry sector can promote the invasion by these species into natural and semi-natural habitats surrounding plantations (Essl et al., 2010; Richardson and Rejmánek, 2011).

Natural regeneration involves many stages in the life cycle of plants, with seedling establishment recognized as a critical step for its success (Castro et al., 2004; Houle, 1996; McAlpine and Jesson, 2008). Young seedlings are more vulnerable to resources availability and stress factors, which in long term, determine species establishment success and distribution (Niinemets and Valladares, 2006). Light and soil moisture conditions have been considered as the main drivers of plant distribution (González-Muñoz et al., 2011; Niinemets and Valladares, 2006). Indeed, drought summer in the Mediterranean region is highly limiting for plant performance and recruitment (Chytrý et al., 2008b).

It´s well known that the success of establishment of a new species is context specific, and depends of both the characteristics of the new species itself and of the existing native vegetation as well habitat conditions (Lonsdale, 1999; Rejmánek et al., 2005). In turn, susceptibility of a receiving community can change in function of competitive and facilitative interactions, nutrient availability or disturbance levels (Davis et al., 2000). Particularly, the role of disturbance have been long recognized as possible important driver of invasion (Elton, 1958). For instance, many studies have observed that disturbance, specifically vegetation removal, favors exotic plants and could be a prerequisite for their establishment (e.g. Alston and Richardson, 2006; Davis and Pelsor, 2001; Davis et al., 2000; Higgins and Richardson, 1998; Mitchell et al., 2006). Although the importance of these plant-plant interaction mechanisms on plant recruitment success, very few studies have evaluated it in context of native and exotic species (e.g. Becerra and Bustamante, 2011; Fernandes et al., 2015; González-Muñoz et al., 2011).

In Portugal, forest plantations are predominantly composed of two species, *Eucalyptus globulus* Labill. (exotic species) and *Pinus pinaster* Aiton (native species) (ICNF, 2013). *E. globulus*, native from Australia, was introduced in the middle of the 19th century and since the middle of 20th century the development of the pulp and paper industries originated the expansion of *E. globulus* plantation in Portugal (Alves et al., 2007). In turn, *P*. pinaster species is the most representative autochthonous species in Portugal, as confirmed by numerous archaeological and geological traces, for at least 33,000 years (Figueiral, 1995). The current pine distribution area had been strongly influenced by human activities and since the 19th century was used in large scale reforestation, which lead to its expansion clearly beyond their natural distribution (Aguiar et al., 2007; Figueiral, 1995). Both species are highly represented in Portugal mainland, which *E. globulus* represent 26% and *P. pinaster* 23% of its forest cover (ICNF, 2013), and as a cultivated trees, its current distribution in country is mainly a result of human activity. These species also have similarities on geographical range, mostly concentrated in the centre and north of the country. Accordingly, one of the major limiting constrains to *E. globulus* and *P. pinaster* natural establishment in Iberian Peninsula is water availability (Almeida et al., 1994; Alves et al., 2012; Catry et al., 2015; Rodríguez-García et al., 2011, 2010; Ruano et al., 2009). Although the ecology and physiology of these species is well known (Correia and Almeida, 2004; Correia et al., 1989; López et al., 2000; Porte and Loustau, 1998; Rodríguez-García et al., 2011; Sabaté et al., 2002; Schwanz and Polle, 2001), little is known about the regeneration capacity of these species under different habitats and ecological conditions. Both species plantations are surrounded by different natural and semi-natural habitats, and their seeds are dispersed to a variety of microhabitats that provide a range of differences in biotic and abiotic conditions affecting seedling emergence, survival and growth, hence influencing establishment success (Castro et al., 2004; Niinemets and Valladares, 2006).

In the light of this, the aim of this study was to evaluate the establishment capacity of *P. pinaster* and *E. globulus* in an array of ecological conditions and identify those conditions that favor or hamper these species establishment that might contribute to their spread capacity. We measured establishment success in terms of seedling emergence and seedling survival capacity as these traits have been found to be related with invasive capacity (Calviño-Cancela and Rubido-Bará, 2013; González-Muñoz et al., 2011; Niinemets and Valladares, 2006). We investigated the effects of disturbance (vegetation removal), habitat type (forest and shrubland) and climate (mesic and xeric conditions) on their seedling emergence and survival using a seed addition experiment. We asked how do the combination of these factors affect the establishment success of these species? This information is crucial to understand the response of these species to environmental conditions and can help us to explain future shifts in these species distribution and natural establishment outside the planted areas.

METHODS

Study sites

Field sites were established along the Atlantic coast in the north (Aveiro, 48°37’N, 8°34’W, 21 m altitude) and south (Setúbal, 38°39’N, 8°36’W, altitude 85 m) of Portugal (Fig. 1). The climate is Mediterranean with an oceanic influence and is classified in the north (Aveiro) as humid - temperate variant and in the south (Setubal) as a sub-humid - warm variant, according to the pluvio-thermic coefficient of Emberger (Quezel, 1977). For simplicity purposes, we will refer to north site and south site as mesic site and xeric site, respectively. Average (30 year average 1980 – 2010) yearly precipitation is 944 mm at the north site (nearest meteorological station 40°38’N, 8°39’W). The south site is drier having average yearly precipitation of 735 mm (nearest meteorological station 38°33’N, 8°53’W). At both sites the precipitation occurs predominantly from autumn to early-spring (October to April) (Fig. 1). Furthermore, mean annual temperature is 15.6ºC and 16.5 ºC at north and south site, respectively. Climatic differences between sites is more evident during summer months (June to August), with drier and hotter summers in the south (Fig. 1B). Precipitation during summer months is 24 mm and 63 mm in the south and north site, respectively. In south site, mean temperature during summer is 22.4ºC with a mean maximum temperature for the hottest month 30ºC (August) (Fig. 1). In turn, mean temperature during summer is 19.8ºC with a mean maximum temperature for the hottest month 24ºC (August) in the north site (Fig. 1).

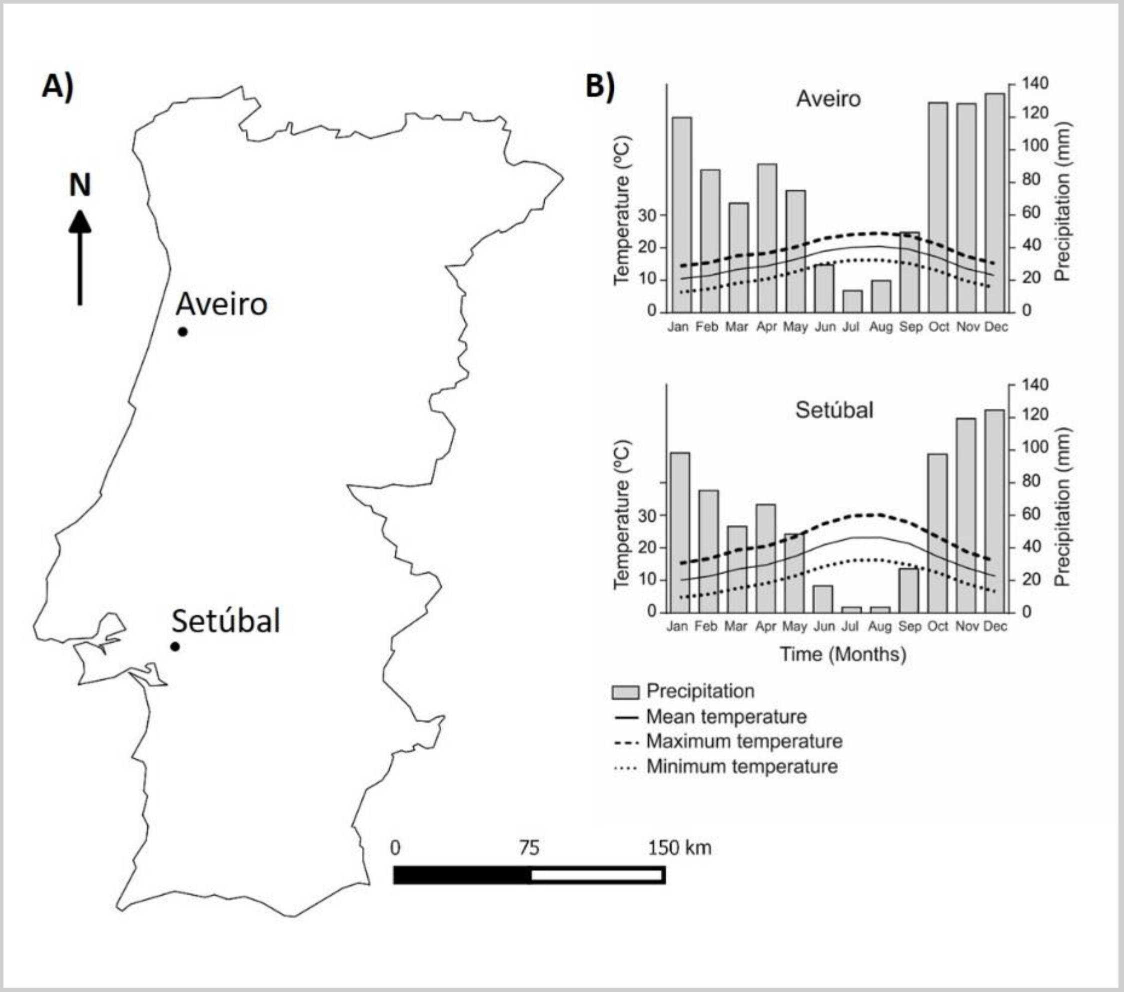


FIGURE 1. Study sites location in Portugal (A), and climatic conditions (30 year average 1980 – 2010) of Aveiro (north) and Setúbal (south) study sites (B).

The vegetation is spatially heterogeneous in both sites, composed of herbaceous, shrub and tree patches. For the study aims we selected two different vegetation communities (habitats) at each study site: native tree patches (forest habitat) and shrublands (see Experimental design). In the north site, study was performed in native pedunculated oak (*Quercus robur*) forest patch and in a near shrubland dominated by short shrubs (around 0.5 m of height) as gorse (*Ulex* spp) and heath (Erica spp.) and also some herbaceous species. In the south site, study was performed in cork oak woodland composed by *Q. suber* and *Q. ilex* (semi-natural forest ecosystem “Montado”) and in adjacent shrubland dominated by rockrose (*Cistus spp.*) and others Cistaceae (such as *Halimium* spp.) and gorse (*Ulex* spp.) These are the most common types of habitats adjacent to *E. globulus* and *P. pinaster* plantations. Plantations of *P. pinaster* and *E. globulus* are present in both sites although no individual of these species was present within the areas of the experiment.

Study species

In both pines and eucalypts, the seeds are found within hard structures (cones in the case of pines and capsules in eucalyptus). The differences in seed weight between species are remarkable; the average weight of the seeds (with coat) was 0.06 g in *P. pinaster* and 0.002 g in *E. globulus* (in this study). Furthermore the thickness of the seed coat is clearly different, with *E. globulus* seeds having a finer coat than those of *P. pinaster*. In *P. pinaster* regular seed production initiates at 10-15 years old (in Iberian Peninsula) and large quantities of seeds are produced (Tapias et al., 2001). *E. globulus* sexual maturity occur earlier than *P. pinaster*, usually at 3-4 years old associated with the change to adult leaves (Jordan et al., 1999) and each capsule contains c. 6-25 viable seeds (Hardner and Potts, 1995; Mimura et al., 2009). Both species seeds show non-dormancy, providing fast germination whenever conditions are favorable (i.e. high water availability and light) (Reyes and Casal, 2001, 1997).

Experimental design

Our experiment was designed to evaluate the effect of disturbance as well as the particular effect produced by different climate regimes (mesic and xeric) and different habitat types (Forest and Shrubland) on *E. globulus* and *P. pinaster* seedling establishment. The potential for seedling emergence and survival of each species was evaluated through a seed addition approach similar to Silva et al. (2011). In each of the two sites, 40 plots of 1m^2 (randomly distributed) were established within the understory of the forests and in shrubland patches (see Study sites). In order to examine the effects of disturbance, plots were randomly left non-disturbed or disturbed through removal of the vegetation layer and soil scarification with a rake, giving a total of 20 plots per disturbance treatment at each habitat type. Of these, ten plots were randomly selected for sowing seeds for each species. In order to avoid seeds viability variations within each species and between species, the seeds used in this study were commercial seeds with the maximum germination rate (99% in both species) under ideal conditions. In each plot, we sowed 60 *P. pinaster* seeds (approximately 4g) or 150 *E. globulus* seeds (approximately 0.4g) which were placed on the ground to simulate the conditions at which seeds are located after natural seed dispersion. To test the effect of different sowing seasons on seedling emergence and survival, seeds were sown in two different dates representing different seasons: spring (March 2013) and autumn (November 2013). Thus, the experiment was replicated (new plots were established) for each sowing season. A total of 320 plots were monitored (two species x two sites x two habitats x two disturbance treatments x two sowing seasons x ten plots). Plots were checked for seedling emergence and survival every 15 days in the first two months after sowing and monthly afterwards, until September 2013 for spring plots and September 2014 for autumn plots. Thus, spring experiment lasted 180 days and autumn experiment lasted 300 days.

Measurements of seedling emergence and survival

In each census, we used a grid with the same total area of the plot ($1m^2$) subdivided in 10 x 10 cm small squares in order to better record newly emerged seedlings at each counting date and distinguish them from new seedlings in subsequent counts (new seedlings were distinguished from survivors). In this way, the temporal duration of survival for each of the seedlings observed was determined based on when each seedling was first observed and when it was last observed (seedling death).

Data analyses

To calculate the final emergence percentage of each plot we used the ratio between the total number of emerged seedlings and total seeds sown. We also assessed the final survival percentage of each plot calculating the ratio between the total number of seedlings that were still alive at the end of the study and the total number of emerged seedlings. We used GLM models (General Linear Model; factorial ANOVA) to determine the effects of sowing season (autumn or spring), site (mesic or xeric), habitat type (forest or shrubland) and disturbance treatment (disturbed or non-disturbed) on final seedling emergence and final seedling survival for each species separately. Both data (ratio values) were arcsine-transformed to meet assumptions of the analysis (normality and homogeneity of variances). Multiple pairwise comparisons of means and differences between species were tested using Tukey HSD post hoc tests (Zar, 1999). Means are reported with standard deviation (± SD).

Survival rates were analysed using a time to event approach, i.e. we modelled the time that the seedlings remained alive (survival times). Seedlings that were still alive at the end of the study were considered censored data, as they may die or not in the future. This approach was also used in other studies (e.g. Calviño-Cancela and Rubido-Bará, 2013; Lorentz and Minogue, 2015; Wassie et al., 2009). The model used for the analyses was Kaplan-Meier survival analyses (Kleinbaum and Klein, 2005) which estimated the survival functions (the probability of a seedling remaining alive at time t) for each factor group. Cox-regression survival analysis (Cox Proportional Hazards) was used to see the interactions among factors explicitly (Kleinbaum and Klein, 2005).

All statistical analyses were performed with the STATISTICA 13.0 package (Dell Inc., Tulsa, OK, USA).

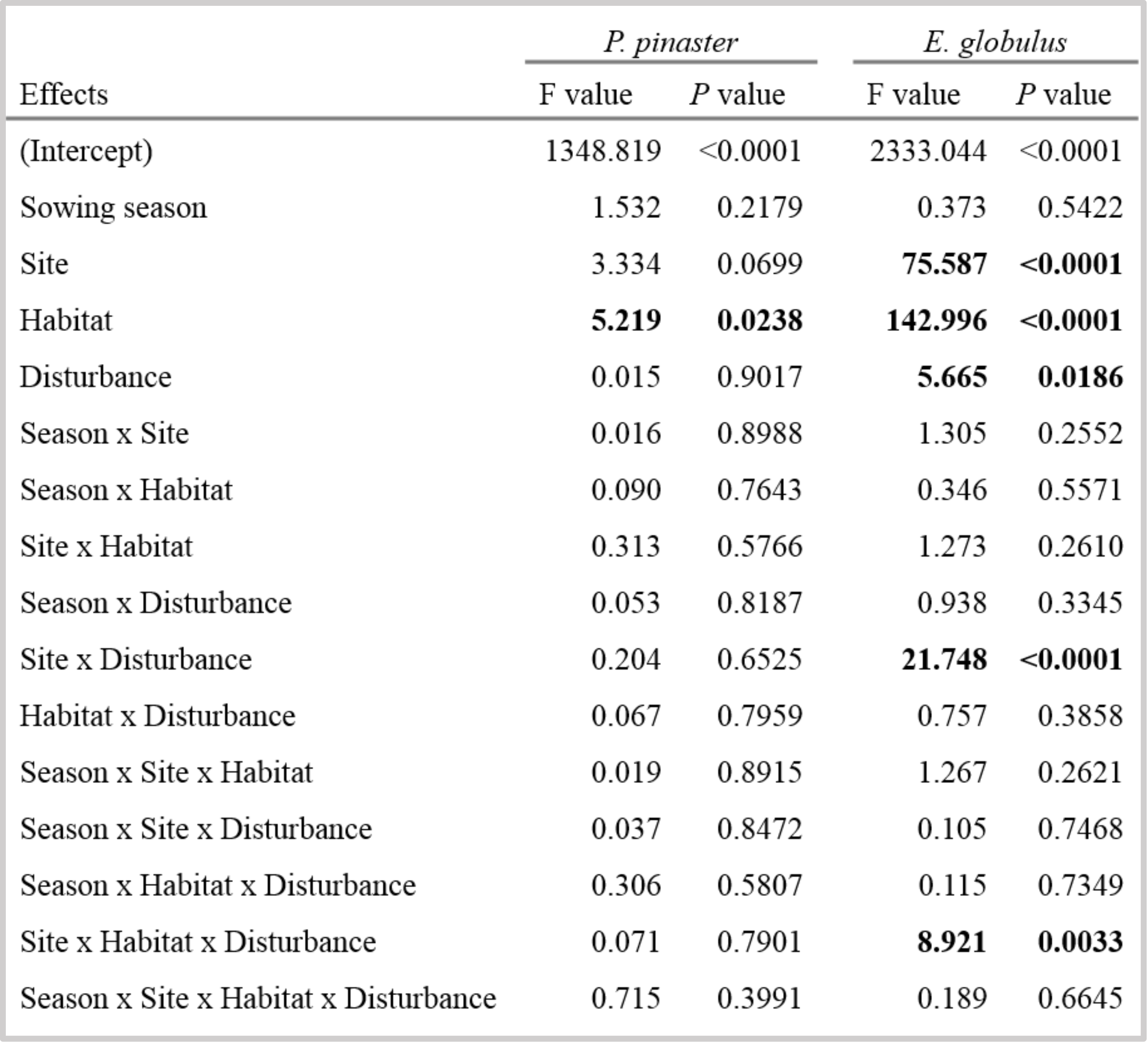
RESULTS

Seedling emergence

We analysed the effects of sowing season, site, habitat type and disturbance in seedling emergence percentage of each species separately (Table 1). The sowing season (spring vs autumn) did not affect seedling emergence in both species (Table 1). For *P. pinaster*, emergence percentage differed significantly between habitat types (Table 1). Specifically, seedling emergence of *P. pinaster* was more limited in the forest habitat than in shrubland habitat (8.50 ± 5.23 vs. 10.88 ± 6.68 %, N=80, *P* = 0.0223). However, we found no significant interactions among factors (Table 1; Fig.2).

E. globulus seedling emergence was affected by site, habitat and disturbance treatments (Table 1). However, we also found a significant statistical interaction between these tree factors (Table 1). Specifically, emergence was significantly higher in the mesic site than in the xeric site although only in the disturbed plots (Fig. 2). In addition, we observed that in both sites seedling emergence was significantly higher in the shrubland than in the forest habitats (Fig. 2). In the mesic site, emergence was higher in the disturbed than in the undisturbed plots although only significantly different in the shrubland habitat (Fig. 2). In turn, in the xeric site we found no significant differences between disturbance treatments (Fig. 2).

TABLE 1. GLM analyses of the effects of sowing season (spring, autumn), site (mesic, xeric), habitat (forest and shrubland) and disturbance (disturbed, undisturbed) on seedling emergence percentage of *P. pinaster* and *E. globulus*. Significant effects are highlight in bold.



	P. pinaster		*E. globulus*	
Effects	F value	*P* value	F value	*P* value
(Intercept)	1348.819	<0.0001	2333.044	<0.0001
Sowing season	1.532	0.2179	0.373	0.5422
Site	3.334	0.0699	**75.587**	**<0.0001**
Habitat	**5.219**	**0.0238**	**142.996**	**<0.0001**
Disturbance	0.015	0.9017	**5.665**	**0.0186**
Season x Site	0.016	0.8988	1.305	0.2552
Season x Habitat	0.090	0.7643	0.346	0.5571
Site x Habitat	0.313	0.5766	1.273	0.2610
Season x Disturbance	0.053	0.8187	0.938	0.3345
Site x Disturbance	0.204	0.6525	**21.748**	**<0.0001**
Habitat x Disturbance	0.067	0.7959	0.757	0.3858
Season x Site x Habitat	0.019	0.8915	1.267	0.2621
Season x Site x Disturbance	0.037	0.8472	0.105	0.7468
Season x Habitat x Disturbance	0.306	0.5807	0.115	0.7349
Site x Habitat x Disturbance	0.071	0.7901	**8.921**	**0.0033**
Season x Site x Habitat x Disturbance	0.715	0.3991	0.189	0.6645

Pairwise comparisons between species showed that *E. globulus* emergence percentage was significantly higher than *P. pinaster* (F = 27.047, $P < 0.001$) although only in shrubland habitat at mesic site (Disturbed plots: 32.83 ± 9.68 vs. 12.08 ± 5.35, N=20, $P < 0.001$; Non-disurbed plots: 19.10 ± 9.26 vs. 11.75 ± 7.20, N=20, $P = 0.0456$; Fig.2).

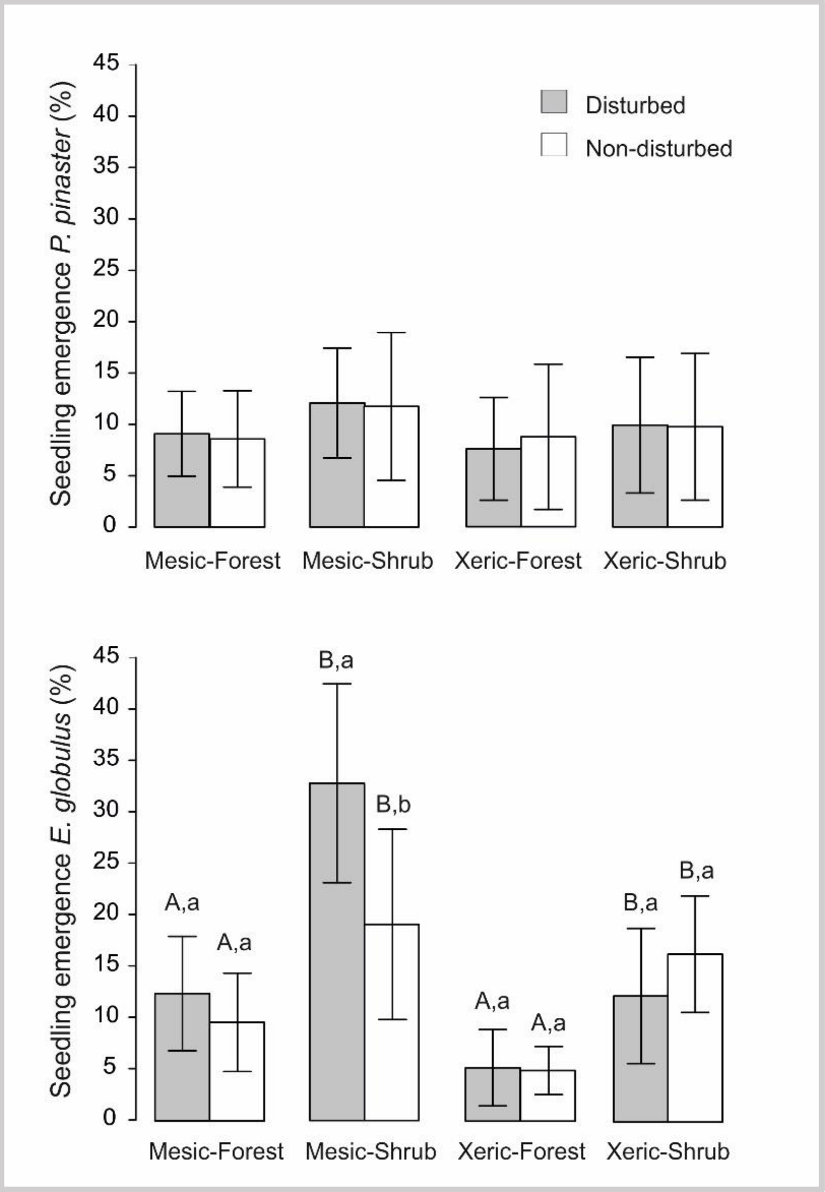


FIGURE 2. Seedling emergence percentage in *P. pinaster* (upper graph) and *E. globulus* (lower graph) species to all combinations of three factors: site, habitat and disturbance. Paired bars depict mean ± 1SD under disturbed and undisturbed treatments, and site (mesic or xeric) and habitat (forest or shrubland) are noted on the *x* axis. N for each bar = 20. Different *lowercase* and *uppercase letters* indicate significant statistical differences (Tukey Post-hoc test, $P < 0.05$) between disturbance treatments within a single habitat and habitats within a single disturbance treatment at each study site, respectively.

Seedling survival

Seedling survival rate, as examined with Kaplan-Meier survival analysis, showed in relation to *P. pinaster* a significant effect of season ($Chi^2 = 59.19$, $P < 0.001$), site ($Chi^2 = 48.13$, $P < 0.001$) and habitat ($Chi^2 = 41.08$, $P < 0.001$). Specifically, we observed that in the spring sowing season mortality rate was significantly higher than in the autumn for the both sites (mesic site: $Chi^2 = 47.65$, P < 0.001; xeric site: $Chi^2 = 15.43$, $P = 0.071$) (Fig. 3). Survival rate was significantly higher in the mesic site than in the xeric site (in both seasons - autumn: $Chi^2 = 47.76$, $P < 0.001$; spring: $Chi^2 = 9.35$, $P = 0.002$; and in both habitat types - forest: $Chi^2 = 28.93$, $P < 0.001$; shrubland: $Chi^2 = 21.68$, $P < 0.001$) (Fig. 3). In both sites, seedling survival rate was significantly higher in shrubland than in forest habitat (mesic: $Chi^2 = 25.14$, $P < 0.001$; xeric: $Chi^2 = 18.49$, $P < 0.001$) (Fig. 3). In addition, interaction between site and disturbance treatment was also significant ($Chi^2_{3df} = 55.34$, $P = 0.007$). Survival rate was significantly higher in the disturbed plots than in the non-disturbed although only in the mesic site (mesic: $Chi^2 = 5.15$, $P = 0.023$; xeric: $Chi^2 = 2.42$, $P = 0.120$). However, we found no significant differences between disturbance treatments within a single habitat at each site (mesic - forest: $Chi^2 = 1.85$, $P = 0.174$; mesic-shrubland: $Chi^2 = 3.75$, $P = 0.063$; xeric - forest: $Chi^2 = 1.07$, $P = 0.301$; xeric-shrubland: $Chi^2 = 2.26$, $P = 0.133$) (Fig. 3).

In relation to *E. globulus*, mortality was very intense in the first two months after emergence (Fig. 3). There was a significant interaction between sowing season and site ($Chi^2_{3df} = 114.8$, $P < 0.001$), between sowing season and habitat ($Chi^2_{3df} = 244.5$, $P = 0.0037$) and between site and habitat ($Chi^2_{3df} = 320.7$, $P < 0.001$). Specifically, we observed that in spring sowing season mortality rate was significantly higher than in the autumn (in the both sites) although only in shrubland habitats (mesic site: $Chi^2 = 6.96$, P = 0.008; xeric site: $Chi^2 = 23.67$, $P < 0.001$) (Fig. 3). In addition, survival rate was significantly higher in the mesic site than in the xeric site although again only in shrubland habitats ($Chi^2 = 94.57$, $P < 0.001$) (Fig. 3). In both sites mortality rate was significantly higher in the forest than in the shrubland habitats (mesic: $Chi^2 = 252.43$, $P < 0.001$; xeric: $Chi^2 = 26.68$, $P < 0.001$). In forests, no survival was observed after 60 and 90 days since emergence in the xeric and mesic site, respectively (Fig. 3).

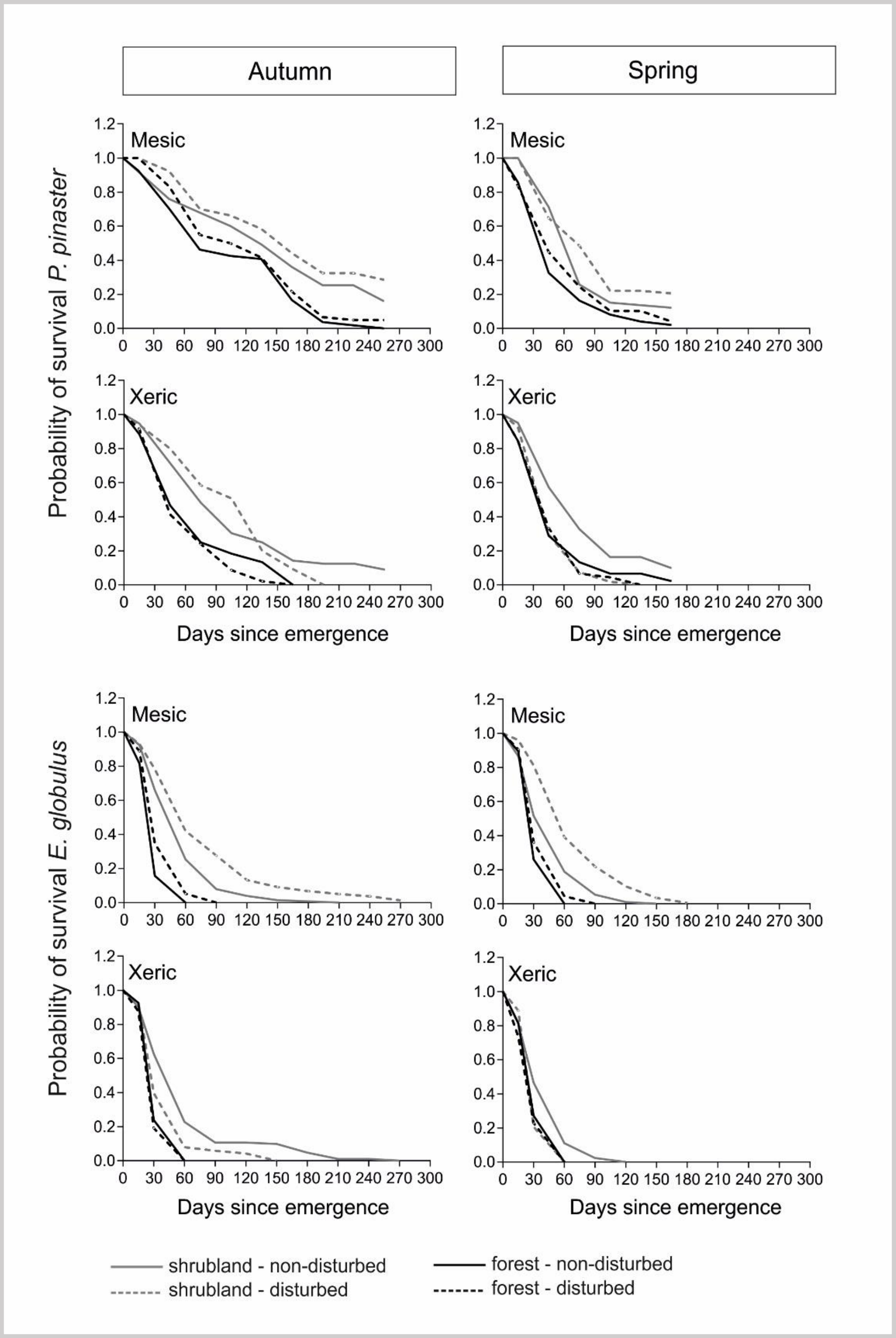
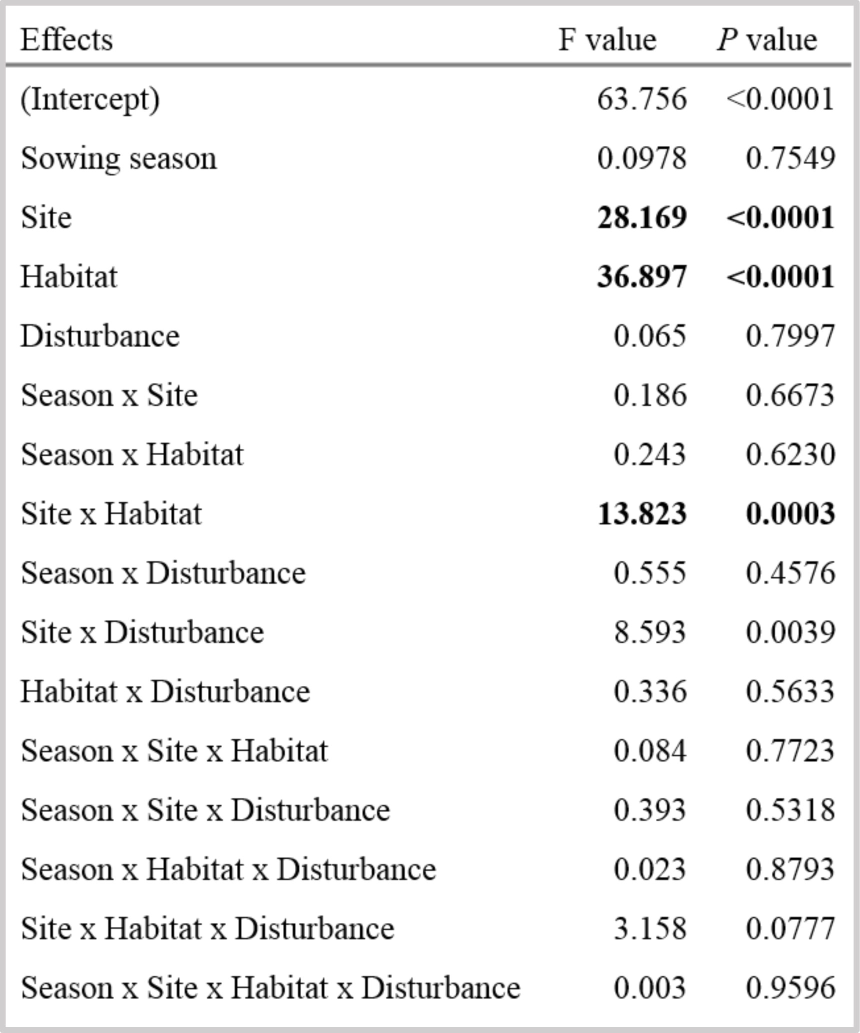


FIGURE 3. Survival curves (Kaplan-Meier survival analysis) of *P. pinaster* and *E. globulus* seedlings emerged from each sowing season (autumn and spring) in the two sites (mesic and xeric) and habitats studied (forests and shrublands). Curves show the probability of seedlings still surviving at different days since emergence in the disturbed and non-disturbed plots at each habitat within each site.

The results also showed that site, habitat and disturbance treatment significantly interacted (Chi^2_{7df} = 410.7, *P* = 0.0026). Specifically, in both habitat types at mesic site survival rate was significantly higher in the disturbed than in the undisturbed plots (forest: Chi^2 = 7.65, *P* = 0.0058; shrubland: Chi^2 = 74.49, *P* < 0.0001) (Fig. 3). In contrast, we found no significant difference between disturbance treatments in the forest habitat at xeric site (Chi^2 = 0.63, *P* = 0.428) but in the shrubland survival rate was significantly lower in the disturbed than in the undisturbed plots (Chi^2 = 22.74, *P* < 0.001) (Fig. 3).

P. pinaster seedling survival percentage differed significantly between sites and between habitats. However, we found a significant interaction between these two factors (Table 2). Specifically, we observed that survival percentage was significantly higher in the mesic than in the xeric site although only in in the shrubland habitat (Fig. 4). In the mesic site seedling survival percentage was significantly higher in the shrubland than in the forest habitats while in the xeric site we found no significant differences between habitats (Fig. 4). In addition, interaction between site and disturbance treatment was also significant (Table 2). We observed that survival percentage was significantly higher in the mesic than in the xeric site although only in the disturbed plots. We found no significant differences between disturbed and non-disturbed plots within a single habitat at each study site (Fig. 4). For *E. globulus*, living seedlings at the end of the study was only found in disturbed plots in the shrubland habitat at mesic site (Fig. 4). Specifically, we registered a final survival of 3 seedlings of the 526 total emerged (0.6%) and 6 seedlings of the 459 total emerged (1.3%) after spring and autumn sowing, respectively. We found no significant differences between sowing seasons (0.62 ± 1.52 vs. 1.47 ± 1.96 % in spring and autumn seasons, respectively; N = 10, *P* = 0.213).

TABLE 2. GLM analyses of the effects of sowing season (spring, autumn), site (mesic, xeric), habitat (forest and shrubland) and disturbance (disturbed, undisturbed) on percent survival of *P. pinaster*. Significant effects are highlight in bold.



Effects	F value	*P* value
(Intercept)	63.756	<0.0001
Sowing season	0.0978	0.7549
Site	**28.169**	**<0.0001**
Habitat	**36.897**	**<0.0001**
Disturbance	0.065	0.7997
Season x Site	0.186	0.6673
Season x Habitat	0.243	0.6230
Site x Habitat	**13.823**	**0.0003**
Season x Disturbance	0.555	0.4576
Site x Disturbance	8.593	0.0039
Habitat x Disturbance	0.336	0.5633
Season x Site x Habitat	0.084	0.7723
Season x Site x Disturbance	0.393	0.5318
Season x Habitat x Disturbance	0.023	0.8793
Site x Habitat x Disturbance	3.158	0.0777
Season x Site x Habitat x Disturbance	0.003	0.9596

Pairwise comparisons between species showed that *E. globulus* seedlings mortality rate was significantly higher than *P. pinaster* (Chi^2 = 209.74, *P* < 0.001) in both sites and habitats, without influence of the sowing season (Fig. 3). Moreover, *P. pinaster* final survival percentage was significantly higher than *E. globulus* (F = 38.562, *P* <0.001). Specifically, we found significant differences between species in the shrubland habitat at mesic site (disturbed plots: 31.12 ± 28.41 vs. 1.04 ± 1.76 %, N = 20, *P* < 0.001; disturbed plots: 21.8 ± 27.96 vs. 0 %, N = 20, *P* < 0.001) and in the disturbed shrubland at xeric site (9.70 ± 12.69 vs. 0 %, N = 20, *P* < 0.001) (Fig. 4). In total, we counted 74 *P. pinaster* seedlings (7.96%) and 9 *E. globulus* seedlings (0.27%) remained alive till the end of the study.

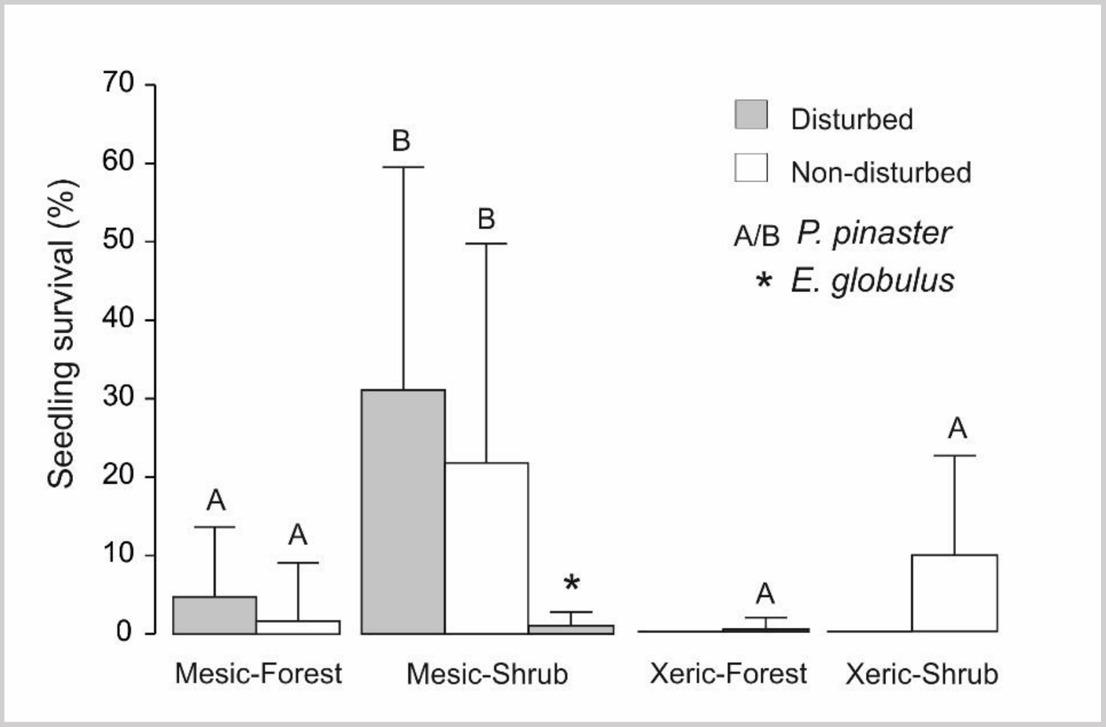


FIGURE 4. Seedling survival (% of living seedlings per plot at the end of the study) of *P. pinaster* and *E. globulus* species in each type of disturbance treatment and habitat type at each study site. Bars depict mean ± 1SD under disturbed (grey) and undisturbed (white) treatments, and sites (mesic or xeric) and habitat type (forest or shrubland) are noted on the *x* axis. N for each bar = 20. Different *letters* indicate significant statistical differences (Tukey Post-hoc test, $P < 0.05$) on *P. pinaster* survival between habitats within a single disturbance treatment at each study site. In all cases the differences between disturbed and non-disturbed plots are no significant. *E. globulus* living seedlings at the end of the study were only registered in disturbed shrubland in the mesic site and their bar is marked by asterisk.

DISCUSSION

It´s well known that water availability from precipitation is one of the most limiting factor for *P. pinaster* and *E. globulus* recruitment (Catry et al., 2015; P. Fernandes et al., 2016; Rodríguez-García et al., 2011). It is worth noting that precipitation in both sites occurs predominantly from autumn to early-spring (October to April) which is coincident with seed sowing dates (November and March) and emergence phase. Thus, soil water availability was not a limiting factor during both sowing seasons even in the xeric site. In accordance, seedling emergence was similar whether seeds were sown in the autumn or spring for both species. Results from Calviño-Cancela and Rubido-Bará (2013) also showed no significant differences on *E. globulus* seedling emergence between spring and autumn sowing seasons, in SW Spain. From seedling emergence phase to establishment phase (survival) air temperatures and water availability decreased, and climatic differences between sites became more accentuated during the summer months. Thus, water availability became more limited as the experiment progressed, mainly in the xeric site. This may explain the significant influence of sowing season on survival rate of both species. In general, seedlings that emerged from spring sowing had higher mortality rate than those emerged from autumn sowing, which is in accordance with the different proximity to summer between sowing seasons. However, at the end of the monitoring period no significant differences on seedlings survival percentage were observed between sowing seasons. Periods with high temperatures and drought accounted for high seedling mortality (many seedlings became brown and dried out during the summer).

Both species recruitment were affected by site conditions and habitat type. Seedlings emergence and survival were limited in the xeric site, with high drought conditions, and under forest habitats (lower light availability). Results obtained by other studies (Catry et al., 2015; González-Muñoz et al., 2011; López et al., 2000; Rodríguez-García et al., 2011) point to the same direction, showing that high soil moisture favored *E. globulus* and *P. pinaster* establishment. Along with the water availability also light has been considered as an important driver in both species establishment. Sánchez-Gómez et al.(2006) showed that *P. pinaster* has poor survival at lower irradiance levels. Calviño-Cancela and Rubido-Bará (2013) showed lower *E. globulus* seedling emergence and survival under pedunculated oak forest than in the shrubland. Indeed, *P. pinaster* and *E. globulus* have been classified as pioneer species, requiring high light regime to establish (Correia et al., 1989; Gil et al., 1990). It´s known that native forests dominated by pedunculated oak, as other broad leaved trees, have low light irradiance regimes at ground level compared to open vegetation of shrubland (Barbier et al., 2008). Therefore, the negative effect of forest on these species recruitment is likely to be caused by a negative shading effect of forest habitat.

However, both species were also influenced by interactive effects of abiotic site conditions and habitat type. Our results suggest that under mesic conditions the negative shading effect by forest canopy on these species establishment can be stronger than the effect of water availability. On the other side, under xeric conditions the effect of drought can be stronger than the effect of light. Indeed, both species seedlings survival were significantly higher in the shrubland than in the forest habitat, although only in the mesic site. Moreover, the survival percentage of these species in the mesic forest was similar to xeric forest, even with higher precipitation in the mesic site. Our results also suggest that in the xeric site the positive effect of higher light availability in the shrubland was not significant on species survival. This may be a consequence of the high mortality rate in the xeric site due to drought conditions. Nevertheless, we found surviving *P. pinaster* seedlings at the end of the study (established) in both sites and under both habitat types (12.4% and 2.8% in the mesic and xeric site, respectively). In contrast all *E. globulus* emerged seedlings died, except in the disturbed shrubland plots at the mesic site although with a very low survival percentage (0.27%).

In spite of the higher seedling emergence, *E. globulus* displayed higher mortality rate during initial phase of seedling growth than *P. pinaster*. Moreover, the survival percentage of *E. globulus* was significantly lower than *P. pinaster* (0.27% vs 7.96%). The larger seeds of *P. pinaster* originated robust seedlings in comparison with very tiny seedlings emerging from *E. globulus* seeds. Likewise, Reyes & Casal (2001) showed that larger seeds give rise to more vigorous seedlings and have a lower mortality rate than seeds of smaller size. Eucalyptus produce very large quantities of seeds but very small seeds with no obvious endosperm and the newly emerged seedlings (sustained by cotyledon photosynthesis) need that their roots penetrate into suitable wet substrate very quickly to survive (Rejmánek and Richardson, 2011). Our results seem indicate that *E. globulus* seeds can successfully emerge when water availability is adequate, but seedlings survival and subsequently establishment is dependent on seasonal fluctuations in water availability during the establishment phase. Indeed, this species is also reported as highly sensitive to drought, particularly at the seedling stage when seedlings are especially vulnerable (Silva et al., 2004). Thus *E. globulus* seedling establishment may be limited by the combination of two factors: 1) small seed size; 2) high vulnerability to drought.

Considering the disturbance effects it was expected that conditions after disturbance, with reduced intraspecific competition (higher water, light and nutrients availability), enhanced seedling emergence and survival (Castro et al., 2004). Microsites created by disturbance can differ greatly in levels of environmental resources and can, therefore have a major influence on the establishment of younger seedlings. However, *P. pinaster* emergence and survival was not significantly affected by disturbance. In agreement with our results, others studies showed that shrub cover did not compete with *P. pinaster* seedlings (Calvo et al., 2003; Rodríguez-García et al., 2011, 2010, 2007) and under drought stress, processes of facilitation may be more frequent that competition

(Rodríguez-García et al., 2010). Indeed, in habitats considered stressful for a target plant, host plant communities could facilitate its establishment through an amelioration of soil drought and high temperatures (Becerra and Bustamante, 2011). This effect may also explain why we found *P. pinaster* seedlings established under non-disturbed plots in the xeric site. Moreover, this species has enormous ecological plasticity, tolerates conditions varying from Mediterranean to Temperate-Oceanic climates (Rodríguez-García et al., 2011). Indeed, *P. pinaster* can be more drought resistant than oak species (Sánchez-Gómez et al., 2006b). From the findings in our study, *P. pinaster* can also be more drought resistant than *E. globulus*. Thus, we have two factors that may have allowed *P. pinaster* establishment under xeric conditions: their drought resistance and the facilitative effect of host plant communities. On the other hand, we observed a positive effect on *E. globulus* establishment by removing vegetation layer, although only in the shrubland habitat at mesic site. Thus, our results suggest the strength of the disturbance effect on *E. globulus* seedling emergence and survival depends on the habitat type and climate conditions. High mortality during initial phase of seedling growth due to unsuitable environmental conditions or competition with other plants have been mentioned by others studies as one of the most important factor that limits eucalyptus colonization (Callaham et al., 2013; da Silva et al., 2011; Rejmánek and Richardson, 2011).

CONCLUSIONS

In conclusion, we found important interactions among site, habitat and disturbance on both species emergence and survival. The effects of drought, light availability and disturbance on these species establishment are context-dependent and can´t be generalized. Both species recruitment were limited in the xeric site and under forest habitats. However, these species were influenced by interactive effects of site and habitat. Under mesic conditions the negative shading effect by forest canopy on these species establishment was stronger than the effect of water availability. On the other hand, under xeric conditions the effect of drought were stronger than the effect of light. In spite of the higher seedling emergence, *E. globulus* displayed higher mortality rate during initial phase of seedling growth than *P. pinaster*. The survival percentage of *E. globulus* was significantly lower than *P. pinaster* (0.27% vs 7.96%). We found surviving *P. pinaster* seedlings (established) in both sites and under both habitat types. In contrast, all *E.*

globulus emerged seedlings died, except in the disturbed shrubland plots at the mesic site although with a very low survival percentage (0.27%). *P. pinaster* had higher plasticity and drought resistance. These results may help to assess the impact of environmental conditions on these species establishment, and can help us to explain future shifts in these species distribution and natural establishment outside the planted areas. Future climatic change scenarios, as droughts will be increasingly longer and more intense and climate irregularity will dominate (IPCC, 2007), will affect these forest species establishment with more drastic effect on *E. globulus.*

ACKNOWLEDGMENTS

This research was funded by Fundação para a Ciência e Tecnologia (FCT) and the Navigator company in the frame of Patrícia Fernandes PhD scholarship from FCT (SFRH/BDE/51709/2011). The authors are grateful to RAIZ - Instituto de Investigação da Floresta e Papel, for logistical support and allowing the establishment of our field sites. Namely, we would like to thank: Alexandre, Carlos Valente and Sofia Corticeiro. We also express our gratitude to all colleagues who helped to perform field work.

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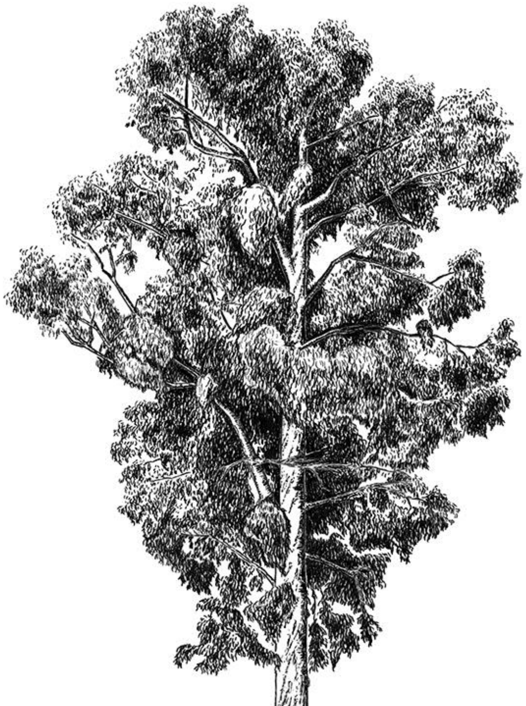
CHAPTER 5

NATURAL REGENERATION IN UNMANAGED EUCALYPT AND PINE PLANTATIONS IN PORTUGAL:

POTENCIAL OF PLANTATIONS IN HARBORING NATURAL VEGETATION



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ABSTRACT

The maintenance of biodiversity in planted forests and, the understanding of the main factors that drive planted species natural establishment are two major challenges for conservationists. The aim of this study was to assess by one hand the value of *P. pinaster* and *E. globluls* forest to harbor natural vegetation and secondly to assess the regeneration potential of these two forestry species under unmanaged plantations. For this propose, we evaluated the recruitment populations of these two planted species and, the understory species richness and cover at three different forest types: (i) *E. globulus* pure plantations; mixed *E. globulus* and *P. pinaster* plantation with eucalypt dominant; and mixed *E. globulus* and *P. pinaster* plantation with pine dominant. In total, 24 stands (8 stands per forest type) were sampled across northern and centre of Portugal. Overall, 33 plant species were recorded across the three forest types. Individuals of *Quercus spp.* were the most abundant non-planted tree genera. Both species richness and shrub biovolume were higher where precipitation was higher and mean maximum temperature of the warmest month was lower. Overall, *E. globulus* recruitment (1257.7 individuals ha^{-1}) was significant lower than *P. pinaster* (1851.9 individuals ha^{-1}). *E. globulus* recruitment was registered at higher densities in the sites with lower temperature seasonality and higher precipitation. *P. pinaster* recruitment was only influenced by forest type. The *P. pinaster* recruitment was significantly lower in the *E. globulus* pure forest than in the two mixed forests.

In conclusion, the forest types considered here had similar potential to harbour native plant species. The high amount of native broadleaves tree seedlings, such as *Quercus spp.*, registered in the understory of the forests may indicate that mature *E. globulus* plantations have considerable potential for conversion into mixed stands with native broadleaved tree species. On the negative side, the presence of invasive tree species, and the shrub biovolume measured here is likely to increase the risk of fire occurrence and their severity. In mixed forests, recruitment of *P. pinaster* was significantly higher than *E. globulus,* even when *P. pinaster* is not a dominant planted species. Natural recruitment of these planted trees were higher in the mature plantations considered here than in the managed plantations considered in a previous study. This show that plantation age and management can have an important role in the magnitude of the natural establishment of these species. Overall, *E. globulus* showed a more sensitive response to climatic variations than *P. pinaster* species.

INTRODUCTION

Planted forests currently represent about 7% of the total forest area world-wide. Contrary to the area of natural forests, which has been declining, planted forests are expanding (FAO, 2010). It´s clear that plantations will occupy an increasing proportion of future landscapes. In some regions, plantations comprise a major proportion of forest area (FAO, 2010). Plantations are established for a variety of reasons including wood production, soil and water conservation, and more recently, carbon sequestration (FAO, 2010). In many countries, plantation forestry consist mostly on a few fast-growing species (Richardson, 1998). Among the most planted forest species worldwide, we can find *Eucalyptus* and *Pinus* species (FAO, 2010). These species contribute significantly to the economic growth of many regions, but may also produce impacts on biodiversity and ecosystems services (Bremer and Farley, 2010). In addition, the expansion of these trees into habitats outside plantations is also a relevant concern for managers and conservationists (Dodet and Collet, 2012; Richardson, 1998). Thus, both the maintenance of biodiversity in planted forests (Bremer and Farley, 2010; Brockerhoff et al., 2013) and, the understanding of the main factors that drive planted species natural establishment and their potential to invade habitats near to their plantations (Richardson and Rejmánek, 2011) are two major challenges for conservationists.

The potential role of tree plantations for biodiversity conservation is controversial. A common perception of plantation forest is that they are ecological deserts that do not provide habitat for valued organisms. However, many studies have documented that plantations themselves can be valuable as habitat for biodiversity (reviewed in Brockerhoff et al., 2008). Biodiversity in planted forests is commonly being compared with biodiversity in natural forests. Therefore, it´s well known that natural forests offer superior habitat for natural vegetation than plantation forest, mainly due their higher habitat diversity and complexity (Lindenmayer et al., 2003). Nevertheless, plantations established on former agricultural or otherwise degraded land may provide significant opportunities for biodiversity conservation (Brockerhoff et al., 2008). In particular, plantation forests are most important in highly fragmented landscapes where they may represent a large proportion of remaining forest habitat, providing corridors between habitats and improving connectivity between native forest patches (Brockerhoff et al., 2013, 2008; Lindenmayer et al., 1999; Wethered and Lawes, 2005). Given the relevance of tree plantations in many regions and their occurrence in the landscape patch matrix, it is important to determine their ability to harbor native biodiversity which would favor the connectivity of the landscape and, with it, the persistence of native populations at a regional scale (Bender and Fahrig, 2005). Different factors are likely to affect the occurrence potential of natural vegetation in plantations. The selected tree species largely determines the ecological conditions in the stand and thereby, its biodiversity (Palik and Engstrom, 1999). Also the frequency of human interventions related to plantation management such as tree thinning, understory removal and soil mobilization operations, could affect the persistence of natural vegetation. These management practices might have more drastic effects than any competitive or allelopathic effects of the planted trees (Atauri et al., 2004). More intensively managed plantations would have lower potential biodiversity because of the higher loss plant species (Brockerhoff et al., 2008). Similarly, has been hypothesized that plantation management practices can also limit the natural recruitment of planted species, functioning as a barrier to their expansion (Fernandes et al., 2016; Larcombe et al., 2013). Moreover, residence time (age) of unmanaged plantations is higher than in managed plantations. Residence time can influence the success of planted species colonization, as the propagule pressure builds over time (Dawson et al., 2009; Pyšek et al., 2009). Because of these plantation management and age-related barriers to natural establishment of planted trees, it´s important to evaluate the recruitment potential of planted species in plantations that are left unmanaged and unharvested (abandoned plantations), where these barriers can disappear. Thus, it is important to specify the management context of the study plantations. Finally, the ecological characteristics of understory species (and species present in surrounding habitats), and other environmental and site conditions including adequate seed sources and climatic conditions are likely to affect the occurrence of understory vegetation (Brockerhoff et al., 2008; Pawson et al., 2013). Indeed, climate has been consider as the main driver of plant distribution (Pearson et al., 2004).

In northern and central Portugal, the most common tree plantations are mono-specific and mixed stands of *Pinus pinaster* Aiton (maritime pine) and *Eucalyptus globulus* Labill. (blue gum eucalypt). Both species are highly represented in Portugal mainland, which *E. globulus* represent 26% and *P. pinaster* 23% of its forest cover (ICNF, 2013), and as cultivated trees, its current distribution in country is mainly a result of human activity. *Pinus pinaster* is a very important timber producing native species in Portugal (Garcia-

Gonzalo et al., 2011b). This species is the most representative autochthonous species in Portugal, as confirmed by numerous archaeological and geological traces, for at least 33,000 years (Figueiral, 1995). The current pine distribution area had been strongly influenced by human activities and since the 19th century was used in large scale reforestation, which lead to its expansion clearly beyond their natural distribution (Aguiar et al., 2007; Figueiral, 1995). *Eucalyptus globulus,* native from Australia, was introduced in the middle of the 19th century and, since the 1960s´ registered a large geographical expansion, which sustained the rise of the Portuguese pulp and paper industry (Alves et al., 2007). Most plantations are mono-specific and mixed stands of *E. globulus* and *P. pinaster* and are privately owned in small holdings (land property are frequently divided into many separate parcels). This land fragmentation is likely to lead to low professionalization and rudimentary management, which is considered far from optimal in terms of productivity. However, it brings the opportunity to study the occurrence of understory plant species and to evaluate the natural recruitment of planted trees under non-existent management, thereby allowing to better isolate the effect of the plantation itself from those caused by management practices.

Although *E. globulus* and *P. pinaster* plantations are the forest types most common in Portugal, neither the natural recruitment of these species nor the potential occurrence of native species in these stands under nonexistence management are known. We evaluated the population recruitment of these two planted species and, the understory species richness and cover at three different unmanaged plantation types: (i) *E. globulus* pure plantations; mixed *E. globulus* and *P. pinaster* plantation with eucalypt as dominant; and mixed *E. globulus* and *P. pinaster* plantation with pine as dominant. Understory plants are used here as biodiversity indicator as their diversity is often correlated with that of other taxonomic groups (Rodrigues and Brooks, 2007). Our aim in this study is to assess, by one hand the value of these forest types to harbor natural vegetation and on the other, to assess the regeneration potential of these two planted forest species, *E. globulus* and *P. pinaster* in order to understand what happens when plantations are left unmanaged.

METHODS

Study area

Based on the forest inventory data performed by one of the major Portuguese forestry association - CELPA florestal, we selected those areas that corresponded to pure *E. globulus* or mixed *E. globulus* and *P. pinaster* stands older than 20 years and left unmanaged. The classification of forest type were based on the total percentage cover of target tree (following the national forestry inventory methodology - IFN 2009; ICNF, 2013): pure stands corresponding to covers of *E. globulus* ≥ 75% cover, and mixed stands to covers < 75 % cover of either *E. globulus* and *P. pinaster* species. The mixed plantations are subdivided in two categories: i) Mixed with *E. globulus* dominant - *E. globulus* is considered a dominant species when it accounted for most of the cover; ii) Mixed with *P. pinaster* dominant - *P. pinaster* is considered a dominant species when accounted for most of the cover. From these potential sites, to minimize differences in environmental conditions (e.g. rainfall, temperature, elevation, and soil type), geographically close stands of these three types were chosen. A total of 24 stands (8 stands per forest type) were selected (Fig. 1).

The average site elevation was 194 m a.s.l (ranging from 47 m to 473 m). The climate is Mediterranean: cool rainy winters and hot dry summers. The average of annual mean temperature of the sampled sites was 15ºC. The annual precipitation was 1129 mm, and ranged from 853 mm to 1336 mm.

Field sampling

Plots were sampled from 14th of October to 19th of November 2015, in the 24 selected forest stands (Fig. 1). In each study stand two 100 m^2 (10 x 10 m) plots were established. In order to assess the edge effect on understory vegetation characteristics and forestry species recruitment, a central plot and at least one plot diverging from the central plot was also established in the edge of the stand. We classified the plot location as interior (in the case of the central plot) or as edge (in the case of plots established in the edge of the stand). In total we sampled 52 plots (24 interior plots and 28 edge plots).

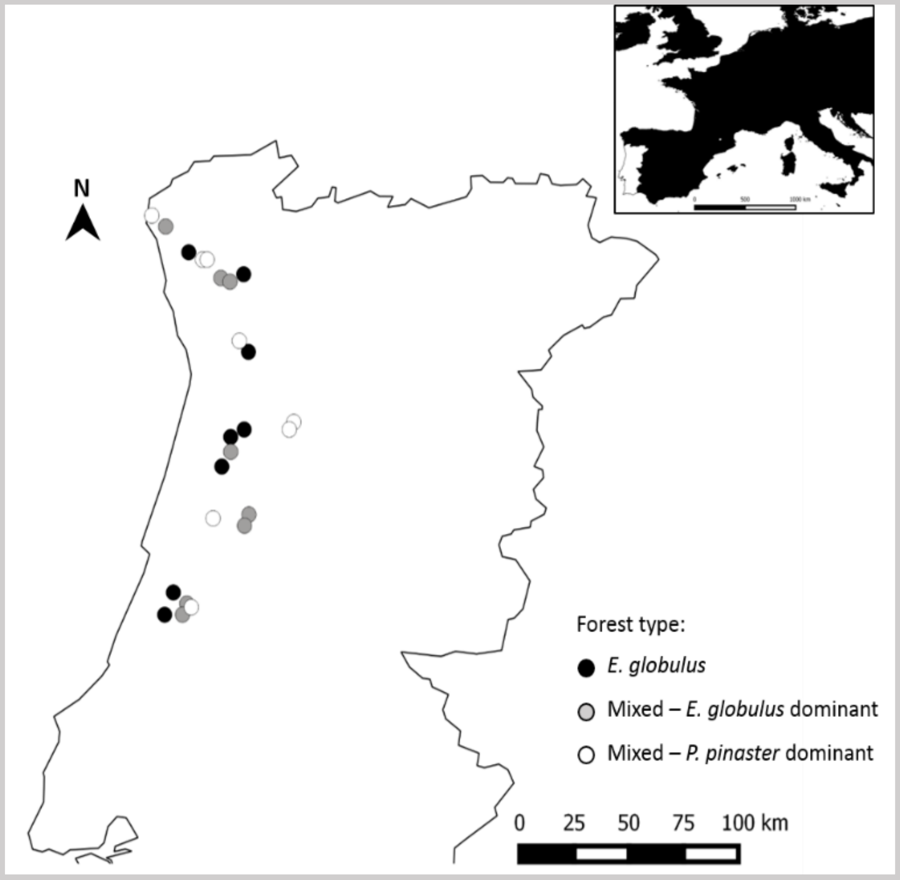

FIGURE 1. Location of the 24 studied unmanaged stands (8 stands for each forest type) across northern and center of Portugal.

We recorded all species present (species richness) in all plots, and measured the height and two perpendicular canopy diameter of each shrub, and afterwards the biovolume was calculated (height x diameter1 x diameter2) (Calviño-Cancela et al., 2012). The sampling months (October and November) corresponded to a period of the year with very low occurrence of herbaceous species. Thus, species richness is underestimated. We only registered the presence of two herbaceous species (*Sinapis alba* and *Oxalis pes-caprae*) with very low occurrence (1.9 %). For trees taller than 1.3 m we measured the circumference at breast height and afterwards the diameter at breast height (DBH) was calculated. In order to characterize the age structure of natural recruitment of *E. globulus and P. pinaster* species, each naturally established individual tree within the sampled plot was afterwards classified to one of four size classes using a combination of height (h) and DBH adapted from Águas et al. (2014) – size class 1: h ≤ 1.3 m; size class 2: h > 1.3 m and DBH ≤ 5 cm; size class 3: h > 1.3 m and 5 < DBH ≤ 7.5 cm; size class 4: h > 1.3 m and DBH > 7.5 cm. In order to assess the potential of *E. globulus* and *P. pinaster* to establish from planted trees, we applied a broad concept of cohort in this study (*sensu*

Schaie, 1984). Accordingly, quantification of natural recruitment of *E. globulus* and *P. pinaster* was obtained from the total plants that had been naturally established from planted trees (i.e. seedlings, saplings and young trees - all plants in the size class 1 to 4).

Data analysis

Data analysis were performed in order to: (i) provide insights on the variations of species richness and structure (biovolume) across the forest types; (ii) analyse the potential recruitment of *E. globulus* and *P. pinaster* under unmanaged plantations; (iii) understanding the relative effect of forest type, plot location and climate explanatory factors on the variations of response variables described above (Table 2). In general, significance of differences across forest types and, between the two target species *(E. globulus vs P. pinaster)* was assessed through the non-parametric Kruskall-Wallis one-way analyses of variance. In case that significant differences were detected ($p < 0.05$), Mann-Whitney U post hoc tests were applied to determine the significance of forest type or target species pair-wise differences. Four response variables were modelled using generalized linear models (GLM): number of *E. globulus* individuals resulting from natural regeneration (*E. globulus* recruitment model), number of *P. pinaster* individuals resulting from natural regeneration (*P. pinaster* recruitment model), total number of species per plot (species richness model) and the total biovolume per plot (biovolume model). The abundance of *E. globulus* and *P. pinaster* resulting from natural regeneration and species richness were modelled using a Poisson distribution, which is typically used for counted data (Gelman and Hill 2007). The biovolume of the understory shrubs was modelled using a gamma distribution. Two categorical explanatory variables were used (Table 1): Forest type and plot location. To account for climate influence, we selected seven climate variables that seemed ecologically most relevant for plant establishment success (i.e. plant growth and survival; Ibáñez et al., 2009) (Table 1). We selected mean annual temperature, mean minimum temperature of the coldest month, mean maximum temperature of the warmest month (these last two variables inform us about the tolerance limits of plants in respect to temperature), temperature and precipitation seasonality (i.e. coefficient of variation in temperature/precipitation along the year), annual precipitation and mean precipitation of the warmest quarter (summer precipitation, which provides a proxy for dryness during summer months). Climate data were averages from 1950-2000 downloaded from the WorldClim data base (Hijmans et al., 2005). Each of the plot centre location was intersected with climate data at a 30 arcsec resolution (~1 km). Prior to modelling we checked the climate variables for collinearity using pair-wise Pearson´s correlation test, excluding correlation values higher than 0.6 and then selected the ones with best explanatory power. Climate variables were highly correlated and thus we selected only tree non-collinear variables: temperature seasonality, mean maximum temperature of the warmest month and annual precipitation (Table 1). All statistical analyses were performed with the R-CRAN software (R Development Core Team 2009).

TABLE 1. Explanatory variables used in the models to predict natural recruitment (nº of plants established by natural regeneration) of *E. globulus* and *P. pinaster*, species richness and understory shrub biovolume (height x diameter1 x diameter2) in the 52 sampled plots.

Variable	Description	Source	Frequency (%)	Mean (SD)	Range
Forest type [a]	Dominant species in the plot	Forest inventory data (CELPA)	*E. globulus* (26.9) Mixed-*E. globulus* dominant (34.6) Mixed-*P. pinaster* dominant (38.5)	-	-
Plot location [a]	Two different locations in the stand: interior and edge		Interior (46.2) Edge (53.8)	-	-
		WorldClim (Hijmans et al. 2005)			
Mean annual temperature (ºC)				14.5 (0.76)	13.2 – 16.6
Temperature Seasonality [a]				4067.8 (312)	3515 - 4727
Mean minimum temperature (ºC January)				5.3 (1.1)	3.6 – 6.8
Mean maximum temperature (ºC July) [a]				25.86 (1.21)	23.2 – 28.4
Annual Precipitation (mm) [a]				1129.1 (166.8)	853– 1336
Precipitation Seasonality				52.9 (1.6)	51 - 56
Summer precipitation (mm)				86.3 (19.9)	54 - 116
Elevation above the sea level (m)				194.04 (120.8)	(47-473)

[a] Variables included in the final analysis to avoid collinearity among continuous variables.

RESULTS

Natural regeneration of plant species in the mature plantations

Overall, 33 plant species were recorded across the three forest types, of which 3 were invasive alien species (Table 2). The most common species were *Ulex europaeus* (in 90.4 % of the plots), *Quercus spp.* (88.5 %), *Pteridium aquilinum* (88.5 %), *Calluna vulgaris* (61.5 %) and *Erica cinerea* (55.8 %). These species occurred in all sampled forest types (Table 2). The occurrence of native plant species were recorded in 65.5 % of the plots, although invasive plant species were also recorded in 17. 3% of the plots. *Quercus species* were the most abundant non-planted tree genera (Table 2). In total, 743 *Quercus* spp. individuals (409 ha^{-1}) were recorded across the three forest types. The higher density of *Quercus spp.* was found under *E. globulus* pure stands, with a total of 228 individuals recorded in 14 sampled plots (1628.6 ha^{-1}). The majority of *Quercus spp.* measured were seedlings (h < 1.3 m), indicating a high regeneration potential under these forest types.

The total number of native plant species (trees, shrubs and herbaceous) per plot varied between 3 and 13 and the average richness was 6.5 (SD = 2.49) species. Forest type did not influence significantly species richness and neither the understory shrub biovolume *(height x diameter1 x diametre2)* (Fig.3).

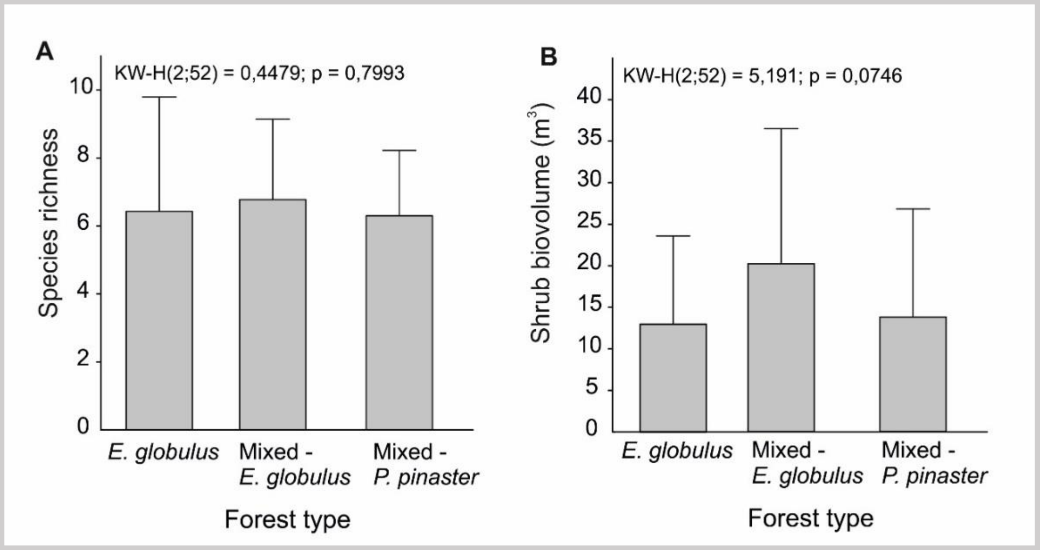

FIGURE 3. Mean values (+SD) of total native species richness (A) and of total shrub biovolume (B) registered in 100 m^2 plots in different forest types: *E. globulus* (N = 14), Mixed forest with *E. globulus* dominant (N = 18) and, Mixed forest with *P. pinaster* dominant (N = 20). Results for Kruskal-Wallis (*H* and *p*) are included in the figure.

TABLE 2. Frequency of occurrence of plant species registered in the 52 sampled plots across tree forest types: pure *E. globulus* (N = 14), Mixed forest with *E. globulus* dominant (N = 18) and, Mixed forest with *P. pinaster* dominant (N = 20). Fourteen species were non-identified. The occurrence of species in each forest type are marked by x.

Species	Frequency (%)	*E. globulus*	Mixed-*E. globulus*	Mixed-*P.pinaster*
Native				
Ulex europaeus	90.4	x	x	x
Quercus spp.	88.5	x	x	x
Pteridium aquilinum	88.5	x	x	x
Calluna vulgaris	61.5	x	x	x
Erica cinerea	55.8	x	x	x
Castanea sativa	28.8	x	x	x
Rubus ulmifolius	26.9	x	x	x
Daboecia cantabrica	21.2		x	x
Pterospartum tridentatum	21.2	x	x	x
Hedera hibernica	19.2	x	x	x
Lithodora prostrata	15.4	x	x	x
Cistus salviifolius	11.5	x		x
Erica ciliaris	9.6	x		x
Genista triacanthos	7.7	x	x	
Rubia peregrina	7.7	x		x
Teucrium scorodonia	7.7	x	x	x
Cistus psilosepalus	3.8		x	
Ruscus aculeatus	3.8	x		
Cistacea sp.	1.9		x	
Cistus monspeliensis	1.9	x		
Erica arborea	1.9			x
Frangula alnus	1.9	x		
Ilex aquifolium	1.9	x		
Myrtus communis	1.9			x
Phillyrea angustifolia	1.9			x
Quercus coccifera	1.9	x		
Salix atrocinerea	1.9	x		
Sinapis alba	1.9	x		
Smilax aspera	1.9	x		
Invasive alien species				
Acacia dealbata	13.5	x	x	x
Acacia melanoxylon	3.8	x	x	
Oxalis pes-caprae	1.9	x		

The GLM analyses showed that these two response variables (species richness and shrub biovolume) were only influenced by climatic factors. Both species richness and shrub biovolume were higher where precipitation was higher and mean maximum temperature of the warmest month (July) was lower (Table 3). However, species richness model and biovolume model explained only 4.9 % and 7.9 % of the deviance, respectively (Table 3).

Natural regeneration of E. globulus and P. pinaster

GLM analyses showed that *E. globulus* recruitment was influenced by forest type (*P* < 0.001) and climatic variables (*P* < 0.001) (Table 3). The recruitment was significantly lower in the mixed forests with *P. pinaster* dominant than in the other two forest types and, no significant differences between *E. globulus* pure forest and mixed forest with *E. globulus* dominant was found (Fig. 4). According to *E. globulus* model, the recruitment was registered at higher densities in the sites with lower temperature seasonality and higher precipitation (Table 3). This model explained 16.5% of the deviance (Table 3). When investigating the factors influencing *P. pinaster* natural recruitment none climatic variable had influence and there was only a significant influence of explanatory variable forest type (Table 3). The forest type explained alone 40.3 % of the deviance (Table 3).

TABLE 3. Generalized linear model (GLM) for each of the modelled response variables: natural recruitment of *E. globulus* and *P. pinaster* – total abundance of individuals in the size class 1, 2, and 3; richness of species; and shrub biovolume. For each response variable, explanatory variables retained in the final model are signalled, as well as their significance (****P* > 0.001; ** *P* > 0.01; * *P* > 0.05), direction of association with response variable (+ or -) and proportion of explained deviance. For forest type, the categories where the likelihood of abundance was higher are indicated.

Variables	*E. globulus* recruitment	*P. pinaster* recruitment	Richness	Biovolume
Forest type	***	***		
E. globulus	+			
Mixed – *E. globulus* dominant		+		
Mixed – *P. pinaster* dominant		+		
Temperature seasonality	- ***			+ ***
Mean maximum temperature			- *	- ***
Annual precipitation	+ ***		+ **	+ ***
Explained deviance	16.5 %	40.2 %	4.9 %	7.9 %

The *P. pinaster* recruitment was significantly lower in the *E. globulus* pure forest than in the two mixed forests (Fig.4). However, no significant differences between the two mixed forest types was found (Fig. 4).

Pairwise comparisons between species showed that *E. globulus* recruitment was significant lower than *P. pinaster* in the mixed forest types (Mixed with *E. globulus* dominant: $P = 0.047$; Mixed with *P. pinaster* dominant: $P = 0.038$) (Fig. 4). In contrast, *E. globulus* recruitment was significantly higher than *P. pinaster* in the *E. globulus* pure forest ($P < 0.0001$) (Fig. 4).

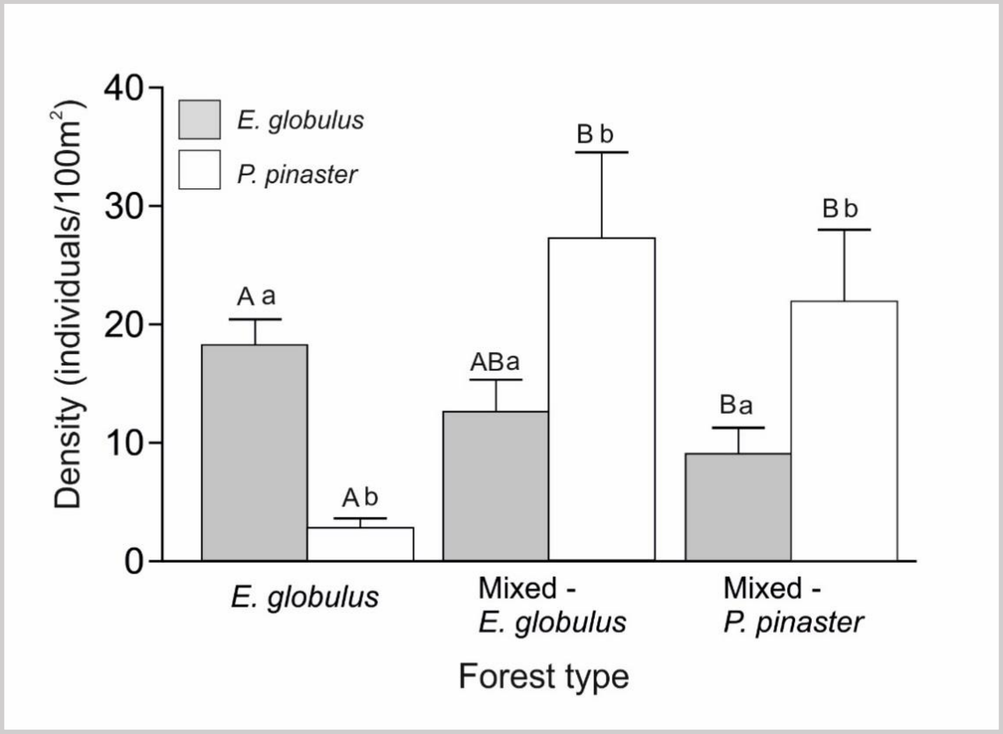

FIGURE 4. Mean values (SE) of total number of new *E. globulus* and *P. pinaster* recruits across forest habitats: *E. globulus* (N = 14), Mixed with *E. globulus* dominant (N = 18) and, Mixed with *P. pinaster* dominant (N = 20). Different *uppercase letters* indicate significant differences ($P > 0.05$) between forest types according to Tukey Post-hoc test based on the recruitment model of each species. Different *lowercase letters* indicate significant differences between two species in each forest type according to Mann-Whitney test.

Overall, *E. globulus* recruitment size class distribution showed higher density of planted trees than natural established individuals of each size class. In contrast, *P. pinaster* displayed a higher relative density of the smallest size class (size class 1) than higher size classes (Table 4). Both of these structural patterns were similar across all forest types (Table 4). The proportion of individuals by natural regeneration in relation to planted trees was higher in *P. pinaster* (963/45 = 21.4 new individuals per planted tree) than in *E. globulus* (654/303 = 2.2 new individuals per planted tree). In total, 957 *E. globulus* individuals were measured. From those *E. globulus* individuals, 303 (582.7 individuals ha^{-1}) were classified as planted trees and the remaining 654 (1257.7 individuals ha^{-1}) were distributed across natural recruitment size classes 1-4 (natural recruitment from planted trees). For each individual forest type, we counted 254 (1814.3 individuals ha^{-1}) natural established *E. globulus* individuals in *E. globulus* pure stands, 223 (1238.9 individuals ha^{-1}) in the mixed forest with *E. globulus* dominant stands, and 177 (885 individuals ha^{-1}) in the mixed with *P. pinaster* dominant stands (Fig. 2). In total, 1008 *P. pinaster* individuals were measured. From those *P. pinaster*, 45 individuals (86.5 ha^{-1}) were classified as planted tree and the remaining 963 (1851.9 individuals ha^{-1}) were distributed across recruitment size classes. Nevertheless, considering only mixed forest types (where *P. pinaster* were planted), 492 (2,733.3 individuals ha^{-1}) and 435 (2,175 individuals ha^{-1}) natural established *P. pinaster* individuals were measured in the mixed with *E. globulus* dominant and mixed with *P. pinaster* dominant, respectively (Table 4).

TABLE 4. Mean (SD) values of DBH and respective range of each of the size classes with the total number of individuals measured (52 sampled plots = 0.52 ha) and their distribution among forest types: pure *E. globulus* (N = 14), Mixed forest with *E. globulus* dominant (N = 18) and, Mixed forest with *P. pinaster* dominant (N = 20). Species are distributed across 4 size classes: 1: h ≤ 1.3 m; size class 2: h > 1.3 m and DBH ≤ 5 cm; size class 3: h > 1.3 m and 5 < DBH ≤ 7.5 cm; size class 4: h > 1.3 m and DBH > 7.5 cm. 52 plots (0.52 ha)

Species	Size class	DBH (cm)		Total nº plants	Nº plants per forest type		
		Mean (SD)	Range		*E. globulus*	Mixed – *E. globulus*	Mixed – *P. pinaster*
E. globulus	1	0.5 (0.1)	0.48 – 1.11	191	45	73	73
	2	2.2 (1.3)	0.48 – 4.77	286	111	93	82
	3	6.2 (0.8)	5.09 – 7.32	72	27	27	18
	4	11.0 (2.2)	7.64 – 15.6	105	71	30	4
	Planted trees	29.8 (12.6)	19.1 – 89.1	303	126	88	89
P. pinaster	1	0.6 (0.2)	0.5 – 2.6	591	8	357	226
	2	2.1 (1.2)	0.5 – 4.9	261	16	91	154
	3	6.0 (0.7)	5.1 – 7.3	48	6	22	20
	4	10.9 (2.1)	7.6 – 13.4	63	6	22	35
	Planted trees	27.2 (6.5)	19.4 – 44.9	45	0	10	35

DISCUSSION

Plantations often support fewer species than natural forests, although under some conditions they can play an important role in biodiversity conservation, particularly at the landscape level, increasing the connectivity for plants and animals populations (Bremer and Farley, 2010). In this study, we evaluated the potential of unmanaged pure *E. globulus*, and mixed *E. globulus* and *P. pinaster* plantations to harbor native plant species. Although the values of the total species found in this study were relatively low when compared to Portuguese primary forests (Proença, 2009; Proença et al., 2010), our results suggested that these mature plantations have potential to harbor native vegetation. In particular, our results showed that native broadleaved tree species, such as *Quercus spp*., *Castanea sativa*, *Frangula alnus*, and *Ilex aquifolium* are colonizing these forests patches, as well many other shrubs species on the understory. Even under pure *E. globulus* plantations we registered high densities of young native broadleaved tree species. This result indicate that mature *E. globulus* plantations have considerable potential for conversion into mixed stands with native broadleaved tree species and pines. Despite differences in methodology (mainly regarding size of sampling plots, ages of plantations and geographical distribution), previous studies have shown similar results, with high levels of understory native tree species in *E. globulus* plantations (Calviño-Cancela et al., 2012; Moreira et al., 2013). On the other side, we have also found that invasive tree species (i.e. Acacia spp.) can occur in mature plantations considered here, being present in 17.3% of the sampling plots.

In addition, forest type was not found to play a significant role in the occurrence of native species. Biodiversity potential is usually expected to be greater in plantations of native than exotic tree species, and in the mixed plantations than in the pure plantations (Brockerhoff et al., 2008). Thus it was expected, in the present case, that pure *E. globulus* forest patches would have a lower species richness than the mixed forests. However, others studies also did not find differences in the potential for native species to occur in plantations dominated by native vs. exotic tree species (Arevalo et al., 2011; Moreira et al., 2013). Specifically, Moreira et al. (2013) did not find significant differences in biodiversity potential between *P. pinaster* and *E. globulus* plantations in Portugal. In short, differences in forest composition and structure between these forest types are not relevant to the potential to harbour native vegetation in our study area. In addition to species richness, our results also showed that forest type did not influence the understory biovolume. Mature plantations with low management, such as those studied here, can support higher amounts of biomass in the understory than intensively managed plantations. A well-developed understory is essential to sustain biodiversity as the base of the food wed (Calviño-Cancela et al., 2012). On the other hand, it´s important to alert that a lush understory in these unmanaged plantations significantly increases the risk of fire and their severity (Fernandes et al., 2004). Thus, it´s important to balance the benefits (i.e. potential to harbour native plant species) and costs (i.e. occurrence of invasive species and increased risk of fire) of the unmanaged plantations. In contrast with the no significant role of forest type, climate was found here to play a significant role in the native species richness and shrub biovolume. Specifically, sites with higher precipitation and lower maximum temperature of the warmest month (July) had higher species richness and shrub biovolume. It´s well know that climate is the main driver of plant distribution from continental to regional scales (Pearson et al., 2004). Indeed, summer drought in the Mediterranean region is highly limiting for plant performance.

Nowadays, expansion of trees into habitats outside plantations is also a concern for managers and conservationists (Dodet and Collet, 2012; Richardson, 1998). Our results showed that in the mixed forest types the regeneration capacity of *P. pinaster* was significantly higher than *E. globulus*, even when *P. pinaster* is not a dominant planted species. A previous study in the managed *E. globulus* and *P. pinaster* plantations pointed at the same direction (Patrícia Fernandes et al., 2016). However, recruitment of both species was markedly lower in the managed plantation than those registered here in unmanaged plantations (102 *E. globulus* ha^{-1} and 557 P. pinaster ha^{-1}; Fernandes et al., 2016). Residence time (i.e. age) influence the success of exotic species colonization due to increased propagule pressure (Dawson et al., 2009; Pyšek et al., 2009). The number of propagules arriving to the new environment (i.e. propagule pressure) has been observed as one of the mechanisms that strongly influence species colonization success (e.g. Catford et al., 2011, 2009; Krivánek et al., 2006; Lockwood et al., 2009; Pyšek et al., 2009; Rouget and Richardson, 2003). Moreover, the management practices present in the industrial plantations, such as periodic clearings of the understory vegetation (both using mechanical methods and herbicides) can also limit the natural regeneration of planted trees. Thus, the higher residence time (i.e. age) of mature unmanaged plantations and the absence of management practices may increase the ability of these planted species to develop a significant propagule pool. The majority of forest paches sampled in our study are surrounded by urban areas and agricultural fields, thus the evaluation of the impact of these high *E. globulus* and P. *pinaster* propagule pressure in their expansion capacity in the natural habitats is extremely difficult.

As found in the previous study about natural regeneration of *E. globulus* and *P. pinaster* (Patrícia Fernandes et al., 2016), the recruitment of *E. globulus* and *P. pinaster* was also influenced by different factors, with *P. pinaster* being independent of climatic variables, while *E. globulus* recruitment was significantly affected by climate variables, showing significantly higher recruitments with higher annual precipitation and lower temperature seasonality. It´s well known that water deficit can compromise *E. globulus* seedling establishment, and survival of young eucalypt (Jacobs, 1955; Stoneman, 1994; Stoneman et al., 1994). Larcombe et al. (2013) found that sites where precipitation seasonality was higher (existence of a rainfall season and accentuated water stress in late summer) had lower probability to have natural establishment of this species, in Australia. Also, Águas et al (2014), Catry et al (2015) and Fernandes et al. (2016) showed that *E. globulus* recruitment is more abundant in areas with higher annual precipitation and lower thermal amplitude, in Portugal. Studies also indicate that water availability from precipitation is the most limiting factor for *P. pinaster* recruitment (Rodríguez-García et al., 2011, 2010; Ruano et al., 2009). However, *P. pinaster* Iberian populations show elevated levels of genetic variability that has enable local adaptation to ecological conditions (Alía et al., 1996) and is known that this species present an enormous ecological plasticity, tolerating a large number of climatic conditions, from Mediterranean to Temperate-Oceanic climates (Rodríguez-García et al., 2010). Thus, we may consider that in our study, the climatic amplitudes in may not be large enough to drive significant changes in *P. pinaster* recruitment.

CONCLUSIONS

We found that mature pure unmanaged *E. globulus* forests and unmanaged mixed forests of *E. globulus* and *P. pinaster* in the northern and central Portugal had similar potential to harbour native plant species. The high amount of native broadleaves tree seedlings, such as *Quercus sp*., registered in the understory of the sampled forests may indicate that mature *E. globulus* plantations have considerable potential for conversion into mixed stands with native broadleaved tree species. On the negative side, we also found that invasive tree species (i.e. Acacia spp.) can occur in mature plantations considered here, being present in 17.3% of the sampling plots, and the shrub biovolume measured here is likely to increase the risk of fire occurrence and their severity. Thus, these characteristics considered here require careful consideration when planning landscape management.

In mixed forests, recruitment of *P. pinaster* was significantly higher than *E. globulus*, even when *P. pinaster* is not a dominant planted species. This indicate that mixed *E. globulus* and *P. pinaster* forest are likely to convert to forests dominated by *P. pinaster*. Natural recruitment of these planted trees was higher in the mature unmanaged plantations considered here than in the managed plantations considered in a previous study (Fernandes et al., 2016). This shows that plantation age and management can have an important role in the magnitude of the natural establishment of these species. Based on models results, it is not clear how *P. pinaster* recruitment is affected by climatic conditions. On the other hand, the recruitment of *E. globulus* was mainly affected by annual precipitation and temperature seasonality. *E. globulus* recruitment was found to be higher in areas with higher precipitation and lower seasonal differences. Considering future climatic changes for the Mediterranean basin, with intensification of dry conditions (increase of maximum summer temperatures and decrease of annual precipitation) and accentuated seasonal temperatures differences (IPCC, 2007), natural recruitment of *E. globulus* will likely become harder. Based on that, *E. globulus* recruitment showed a more sensitive response to climatic variations than *P. pinaster* species.

ACKOWLEDGEMENTS

This research was funded by Fundação para a Ciência e a Tecnologia (FCT) and the Navigator Company in the frame of Patrícia Fernandes PhD scholarship from FCT (SFRH/BDE/51709/2011). We would like to thank CELPA florestal for providing access to plantations location and data.

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CHAPTER 6

GENERAL DISCUSSION
&
FINAL REMARKS

GENERAL DISCUSSION

The main objective of this thesis was to study natural regeneration and expansion capacity of *P. pinaster* and *E. globulus* into adjacent natural habitats to plantations. The analysis of the role of climatic variables, plantation characteristics, habitat type, vegetation cover, and disturbance level aimed to understand the main factors that drive these species natural establishment at different spatial scales. Along the previous chapters, several specific questions linked with this subject were addressed and major conclusions discussed. This section presents a synthesis of the implications of the major findings of this thesis in the understanding of the natural establishment and expansion capacity of *P. pinaster* and *E. globulus*. Additionally, this chapter also point out some recommendations on how management practices can be optimized in order to avoid an undesirable colonization by these species.

Recruitment and spread capacity from forestry plantation into natural habitats

In this thesis, and for the first time, the spatial recruitment pattern of the forestry species *Pinus pinaster* and *Eucalyptus globulus* from plantations into natural habitats was characterized (Chapter 2). Results showed that seedling recruitment was higher close to the plantation and decreased exponentially from the plantation edge to adjacent habitats, in both *E. globulus* and *P. pinaster* (Fig. 1; Chapter 2). This recruitment pattern followed the Hubbell model (Hubbell, 1980) which proposes that since seed density is very high close to the parent plant, recruitment is higher there. Typically, in wind-dispersed species (such as considered species here), seed rain density decreases as a function of distance from the parent plant (Houles, 1995). However, species presented very different recruitment characteristics, regarding the amount of established seedlings and the distance of colonization, much higher for *P. pinaster* (Fig. 1; Chapter 2). The higher *P. pinaster* seedling recruitment (*P. pinaster*: 247 seedlings/ha; *E. globulus*: 22 seedlings/ha), the smallest decline in seedlings density with distance from plantation boundary (*P. pinaster* = -0.036 seedlings/m vs *E. globulus* = -0.048 seedlings/m), and the longer distance of seedling establishment, demonstrated clearly that *P. pinaster* is more successful colonizer of the natural habitats near plantations than *E. globulus* (Chapter 2). The majority of the *E. globulus* recruited seedlings were located less than 15 m from plantation edge (92% of the total seedlings recorded) and were recorded to a maximum distance of 60 m from plantation edge. In contrast, *P. pinaster* seedlings were mostly observed outside of the stands as far as 105 m away (69% of the total seedlings were registered after the plantation boundary) (Chapter 2). Moreover, recruitment of *P. pinaster* occurred in 96% of transects, and *E. globulus* in 32% (Chapter 2). Thus, population expansion was substantially more evident in *P. pinaster*, accomplished by establishing outlying populations and accelerated expansion rate. These results of *P. pinaster* natural establishment and spread capacity confirmed what have been demonstrated earlier on by several authors, particularity in southern hemisphere (Higgins and Richardson, 1999; Rouget and Richardson, 2003).

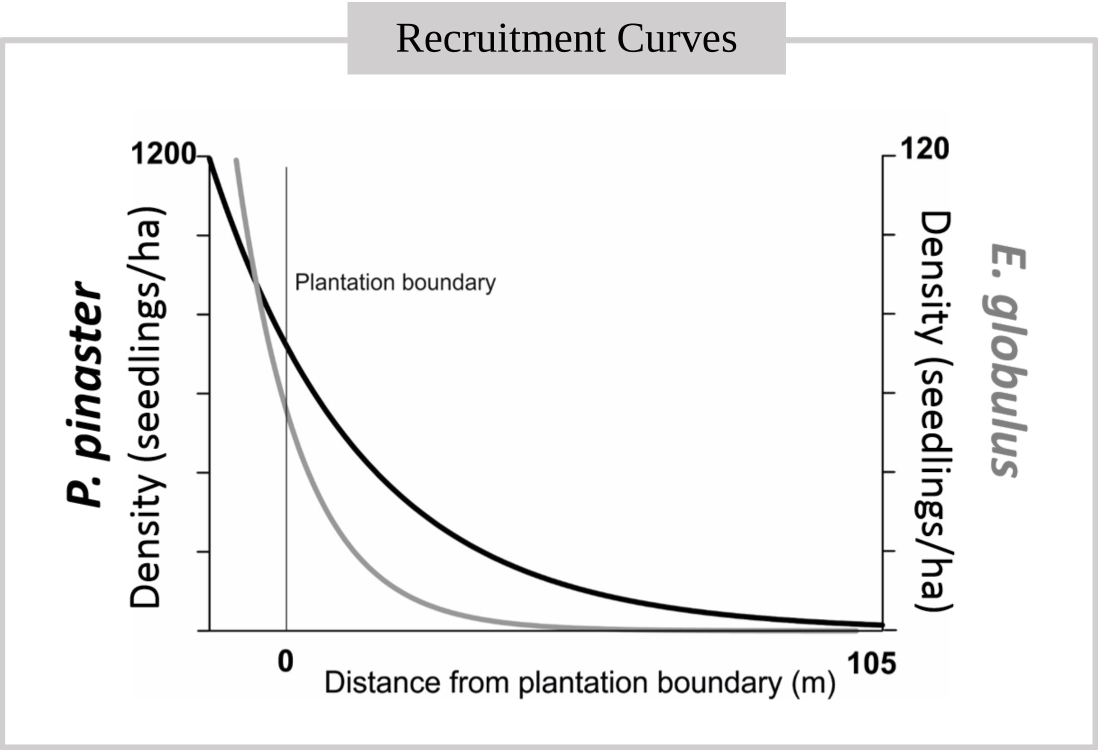

FIGURE 1. Schematic diagram of *P. pinaster* (black line) and *E. globulus* (grey line) recruitment curves. *P. pinaster* average recruitment density is represented in the left Y axis and *E. globulus* average recruitment density in the right Y axis (different scales were used for *P. pinaster* and *E. globulus*).

One the main reason for this discrepancy in species spread capacity may be related with their different seed dispersal mechanism. The two species have very different seed dispersal abilities: *P. pinaster* seeds have large wings as a specialized wind dispersal structure and can be dispersed at long distances; *E. globulus* seeds have no adaptation for dispersal mechanism (wings or fleshy tissues) and are mainly dispersed within the capsules, and capsule dispersal by wind is presumed to be limited (Calviño-Cancela and Rubido-Bará, 2013; Rejmánek and Richardson, 2011). These species are not dispersed by animals. Thus, it appears that one of the main constrains for the spread of *E. globulus* outside plantation sites is their limited seed dispersal, as suggested by other studies (Calviño-Cancela and Rubido-Bará, 2013; Rejmánek and Richardson, 2011; Ritter and Yost, 2009).

Moreover, natural recruitment of *P. pinaster* was also significantly higher than *E. globulus* in mature mixed plantations (unmanaged plantations), even when *P. pinaster* was not the dominant planted species (Chapter 5). This indicate that mixed *E. globulus* and *P. pinaster* forests are likely to convert to forests dominated by *P. pinaster*. Other factors, related with species seedling establishment ability and drought resistance, were investigated in Chapter 4, and may help us to understand the discrepancy in the recruit seedling densities between these species showed in Chapter 2 and 5. In Chapter 4 we measured the establishment success in terms of seedling emergence and seedling survival capacity as these traits have been found to be related with invasive capacity (Calviño-Cancela and Rubido-Bará, 2013; González-Muñoz et al., 2011; Niinemets and Valladares, 2006). We found that, in spite of the higher seedling emergence, *E. globulus* displayed higher mortality rate during initial phase of seedling growth, when compared to *P. pinaster*. Moreover, survival percentage of *E. globulus* was significantly lower than *P. pinaster* (0.3% vs 8% of established seedlings). We found surviving *P. pinaster* seedlings (established) in both studied sites (mesic and xeric sites), and under both forest and shrubland habitats (12.4% and 2.8% in the mesic and xeric site, respectively). In contrast, all *E. globulus* emerged seedlings died, except in the disturbed shrubland plots at the mesic site although with a very low survival percentage (0.3%). Thus, *P. pinaster* seedlings have higher survival capacity than *E. globulus* (Chapter 4). Again it seems that seed characteristics can help to explain this difference. The larger seeds of *P. pinaster* originated robust seedlings in comparison with the very tiny seedlings emerged from *E. globulus* seeds. Likewise, Reyes and Casal (2001) showed that larger seeds give to more vigorous seedlings and have a lower mortality rate than seeds of smaller size. Eucalypts produce very large quantities of seeds but very small seeds with no obvious endosperm, and the newly emerged seedlings (sustained by cotyledon photosynthesis) need that their roots penetrate into suitable wet substrate very quickly to survive (Rejmánek and Richardson, 2011).

Invasive plant species are defined as exotic plants that produce reproductive offspring, often in very large numbers, at considerable distances from the parent plants, and thus have the potential to spread over a large area (Pyšek et al., 2004; Richardson et al., 2000). In contrast, results that support this thesis showed that *E. globulus* has a localized recruitment close to plantation boundaries and very low levels of establishment. *E. globulus* can establish with some regularity within planted stands, and this suggests that the species is becoming naturalized (exotic plant that sustain self-replacing populations without direct human intervention by natural recruitment capable of independent growth; Pysek et al. 2004) in Portugal. However, there were no indications that *E. globulus* is spreading rapidly, or in large numbers, away from the boundaries of plantations into natural habitats as it is characteristic of invasive species. Thus, quantitative results discussed here are in profound disagreement with the classification of *E. globulus* as a major invader in Portugal by Marchante et al. (2014) based on the Australian Weed Risk Assessment (mainly based on species biological traits).

The main factors that drive the natural regeneration

Conceptual models that explain introduced plants establishment have pointed to the combination of having enough propagule pressure and a suitable environment, biotic and abiotic (Catford et al., 2009; Chytrý et al., 2008b). In this thesis, climate was used to estimate abiotic suitability (Walther et al., 2009; Chapter 2, 3, 4 and 5), plantation characteristics as a proxy to propagule pressure (Pyšek et al., 2009; Chapter 2, 3 and 5), and site characteristics (including habitat type) mainly representing the characteristics of the host community (biotic and abiotic suitability) (Rejmanek et al., 2005; Chapter 2, 3, 4 and 5).

The better climate predictors explaining *E. globulus* recruitment were temperature seasonality and annual precipitation. *E. globulus* was more likely to be present in areas with low temperature seasonality and high annual precipitation (Chapter 2; 3; and 5). Previous studies pointed at the same direction (Águas et al., 2014; Catry et al., 2015; Larcombe et al., 2013). Indeed, drought has a strong negative effect on *E. globulus* seedling emergence and survival (Humara et al., 2002; López et al., 2000; Chapter 4). In Chapter 4, we showed that water deficit can compromise summer survival of *E. globulus* seedlings. On the other hand, it was not clear how *P. pinaster* recruitment is affected by climatic conditions. Based on the models explored in Chapters 2 and 5, no climate variable explained *P. pinaster* recruitment. Iberian *P. pinaster* populations show high level of genetic variability that has enable local adaptation to ecological conditions (Alía et al., 1996; Figueiral, 1995) and is known that this species has enormous ecological plasticity, tolerates conditions varying from Mediterranean to Temperate-Oceanic climates (Rodríguez-García et al., 2010). This is also in accordance with results found in Chapter 4, which showed that *P. pinaster* seedlings had higher plasticity and drought resistance than *E. globulus*. These facts could explain why climate variables did not significantly influenced *P. pinaster* recruitment. The climate range among sites sampled in Chapter 2 and 5 could have been insufficient to affect *P. pinaster* recruitment. Based on that, *E. globulus* seedlings establishment showed a more sensitive response to climatic variations than *P. pinaster* species. Future climatic change scenarios for the Mediterranean basin dictate that droughts will be increasingly long and more intense, seasonal temperature differences will be accentuated, and climatic irregularity will dominate (IPCC 2007). These factors will surely affect these forest species recruitment, with a more drastic effect on *E. globulus*. The results that support this thesis showed that *E. globulus* is high sensible to dry conditions (high temperatures and low annual precipitation). Thus, considering future climatic changes for the Mediterranean basin, the natural recruitment (seedlings survival) of *E. globulus* will likely become harder.

Recruitment models performed in this thesis reveled that plantation characteristics (age and area) had no significant influence on *P. pinaster* (Chapter 2) and *E. globulus* (Chapter 2 and 3) recruitment. Intensive management of these plantations (mainly *E. globulus*) could explain these results, since the lower ages of managed plantations might contribute to diminish their ability to develop a significant propagule pool. The results showed in Chapter 5 seem to corroborate this hypothesis. In the mature plantations (older than 25 years and left unharvested after optimal age; Chapter 5) we found a higher recruitment of these species than in the industrial plantations (Chapter 2). Thus, the importance of plantation characteristics might increase in abandoned plantations, as the propagule pressure builds with time.

Finally, site characteristics (such as habitat type, vegetation cover, and disturbance) were the most important group of variables influencing *P. pinaster* and *E. globulus* natural recruitment outside planted areas in comparison to climate and plantation characteristics (Chapter 2 and 3). The identity of habitat (forest, grassland or shrubland) was one of the most important factors explaining these species recruitment. Previous studies also found habitat type to be the foremost factor explaining differences in plant colonization potential (Chytrý et al., 2008b; Gassó et al., 2012). Forest was the most resistance habitat to these species establishment (Chapter 2, 3 and 4). Along with the water availability also light has been considered as an important driver in both species establishment. Sánchez-Gómez et al.(2006) showed that *P. pinaster* has poor survival at lower irradiance levels. Calviño-Cancela and Rubido-Bará (2013) showed lower *E. globulus* seedling emergence and survival under pedunculated oak forest than in the shrubland. Indeed, *P. pinaster* and *E. globulus* have been classified as pioneer species, requiring high light regime to establish (Correia et al., 1989; Gil et al., 1990). It´s known that native forests dominated by oak species (main forest type surveyed), as other broad leaved trees, have low light irradiance regimes at ground level compared to open vegetation of shrubland (Barbier et al., 2008). Therefore, the negative effect of forest on these species recruitment is likely to be caused by a negative shading effect of forest habitat (Chapter 4). In the case of *E. globulus* also grasslands were very resistant to their establishment (Chapter 2 and 3). Grasslands normally show high seasonal fluctuations in water soil and nutrients availability (Rundel et al., 2014). These factors could affect negatively eucalypts seedling survival (Rejmánek and Richardson, 2011; Chapter 4). High mortality during initial phase of seedling growth due to unsuitable environmental conditions or competition with other plants have been demonstrated as one of the most important factor that limits eucalypts colonization (Callaham et al., 2013; da Silva et al., 2011; Rejmánek and Richardson, 2011; Chapter 4). Indeed, survival of eucalypts seedlings are comprised in dense vegetation sites (Chapter 3 and 4). Results from Chapter 3 analysis showed that vegetation cover alone explained more than 47% of eucalypts occurrence variability and was found a negative association between vegetation cover and eucalypt occurrence and density. The same pattern was found in relation to vegetation disturbance level, which was the most important factor explaining eucalypt density. In Chapter 3 was found that although it is more likely to find at least one eucalypt under low shrub cover, highly disturbed areas are hubs for eucalypt density. The eucalypts density found in all high disturbed plots close to plantations (mean = 293 plants ha^{-1}; Chapter 3) is quite similar to the mean density calculated by Catry et al. (2015) in roadside transects adjacent to eucalypt plantations (277 plants ha^{-1}), in Portugal. In spite of the different survey methods, both studies have surveys adjacent to plantations and roadsides can be considered a high disturbed environment with high percentage of bare ground. However, the observed average eucalypts density in both studies is very low compared to well-known invasive forestry species (e.g. pines, Richardson et al., 1994) and also with native maritime pine planted in Portugal (892 plants ha^{-1}; Chapter 2). In Catry et al. (2015), eucalypts presence was found in 60% of the roadside transects adjacent to eucalypt plantations. On the contrary, we only found establishment in 8% of the total plots (Chapter 3). The characteristics of roadsides (usually have high percentage of exposed soil without vegetation) seem to increase the suitability of community to forestry species establishment, increasing the window of opportunity for tree seeds to reach suitable sites. Thus, the low establishment of eucalypts registered in the current study (Chapters 2 and 3) shows that eucalypt density registered in the roadsides by Catry et al. (2015) can´t be generalized to other habitats adjacent to plantations. In addition, Catry et al. (2015) road surveys might have overestimated eucalypt densities as it is difficult to distinguish eucalypts seedlings from vegetative regeneration of larger trees without in-situ observation.

The effect of disturbance on *E. globulus* establishment seems to be context-dependent. In Chapter 4, the positive effect on *E. globulus* establishment by removing vegetation layer was only registered in the shrubland habitat at mesic site. In contrast, *P. pinaster* seedling emergence and survival was not affected by vegetation disturbance (Chapter 4) and all open habitat types studied revealed to be suitable to *P. pinaster* natural regeneration (Chapter 2 and 4). In agreement with these results, others studies showed that vegetation cover did not compete with *P. pinaster* seedlings (Calvo et al., 2003; Rodríguez-García et al., 2011, 2010, 2007). In addition, the effects of drought, light availability and disturbance on these species seedling survival seems to be context-dependent (Chapter 4). Specifically, under mesic conditions the negative shading effect by forest canopy on these species establishment was stronger than the effect of water availability. On the other hand, under xeric conditions the effect of drought was stronger than the effect of light.

Management guidelines

It is important to consider the spatial dynamics of landscape patches for better manage landscape characteristics such as connectivity or permeability in relation to movement of key species (such as planted species) (Hobbs et al., 2014). Hobbs et al. (2014) advanced the idea of “Managing the whole landscape” in situations where we need to deal with mixtures of natural ecosystems that are substantially altered by humans. Following their idea, research studies as the present thesis, have crucial importance to ensure landscape functionality (productivity and conservation).

The high importance of site characteristics in this study reflects that most of the variability in these species establishment occurs at small spatial scales (site scale; < 1km) (Milbau et al., 2009; Chapter 2, and 3). Factors affecting species distribution most likely do so in a hierarchical manner, with different ecological factors affecting plant establishment success more strongly at different spatial scales (a hierarchical framework; Milbau et al., 2009). For example, Pearson et al. (2004) studied the impact of climate and land cover changes on species distributions in Britain and discovered a dominant effect of climate measured at the European scale over land-cover data at the British scale. Although, at smaller scale, the incorporation of land-cover improved the accuracy of the species distribution models, compared what had been shown at the coarser European scale. In accordance with this, climate might control variability in forestry species establishment among plantations (regional scale: 2,000-200 km; Chapter 2, 3, 4 and 5), while site characteristics influence establishment within plantations' area (site scale: 10 – 1,000 m; Chapter 3; and 4). These small-scale factors (vegetation cover, disturbance and habitat type) are essential to precise predictions at more local scales. The climate, higher scale factor, had low explanatory strength on eucalypts establishment (Chapter 3 and 5) and none influence on *P. pinaster* (Chapter 2 and 5). These results are in accordance with the idea proposed by Milbau et al. (2009), suggesting that small-scale factors being better predictors at finer scales if conditions at higher levels are satisfied. In fact, as a cultivated species, *E. globulus* and *P. pinaster* distribution is mainly result of human activity, targeting environmental conditions highly suitable for the species. Thus, monitoring efforts should focus on the site factors determining the colonization: habitat type, ground cover, and disturbance.

Although a native species, the spread of pines outside plantation sites should also be a concern (Taylor et al., 2016). Results from Chapter 2 showed that the colonization of this species from plantations into open habitats (grassland and shrubland) can lead to a conversion of such treeless systems to pine forests in some areas, increasing the fire risk and the impact on more sensible drainage basins, if no monitoring and control practices of natural regeneration outside plantations are implemented. However, monitoring and control practices could be difficult to implement because of the low managements requirements and consequently low human intervention in *P. pinaster* stands. In opposition, industrial *E. globulus* plantations are grown with very short rotations (rotation time in Portugal is 10-12 years) and with a regular basis management, so there are likely to be multiple opportunities for detection and control over the lifetime of a plantation. In addition, limited dispersal ability of *E. globulus* resulted in a localized recruitment close to plantation boundaries which make the implementation of simple management practices, such as maintaining clear firebreaks surrounding the stands, very efficient and would likely greatly reduce the likelihood of their establishment and spread outside plantation sites. From the Chapter 3 findings, we can also suggest that reducing of the eventual disturbance in habitats adjacent to plantations during the management activities would also greatly reduce the natural establishment of *E. globulus*. Considering the differences in spatial pattern of recruitment between species and the different plantation management, it´s evident that the risk of colonization by *E. globulus* will likely be managed more effectively than that of *P. pinaster*. Moreover, the low residence time of *E. globulus* plantations also contribute to diminish their ability to develop a significant propagule pool (Chapter 5). Because of these plantation management and age-related barriers to colonization, plantations that are left unmanaged and unharvested (abandoned plantations) have higher colonization potential, where these barriers disappear (Chapter 5). Sites with accentuated slopes were not considered in this thesis, however both *P. pinaster* and *E. globulus* plantations near drainage lines or stepper slopes need an increased attention because the potential dispersal distances and the difficulty to implement control practices increase in these situations. Taken into account this thesis results, we also suggest the establishment of "sentinel sites" using remote sensing tools (e.g. Google Earth) as described by Visser et al. (2014) or in-situ visits during conventional management as described in Chapter 2. This monitoring system could, over time, produce very valuable data to improve our understanding of forestry species colonization dynamics. It could also alert managers to situations where some form of management intervention is needed, helping, in way, to introduce more professional planning and management into Portuguese pine and eucalypt plantations.

FINAL REMARKS

In conclusion, the results that support this thesis suggest that the colonization outside plantation by *E. globulus* will likely be remarkably slow when compared to *P. pinaster* (species with long-distance seed dispersal and higher seedling survival capacity). The localized recruitment and lower levels of establishment, and the success of native species recruitment under eucalypt forests suggested that currently, *E. globulus* does not demonstrate an invasive behavior in adjacent natural habitats to the plantations. *E. globulus* limited expansion ability seems to be related with the combination of two characteristics: limited seed dispersal, and high seedling mortality rate. In contrast, *P. pinaster* had higher seed dispersal ability, higher ecological plasticity, and higher drought resistance.

The best climate predictors explaining *E. globulus* recruitment were temperature seasonality and annual precipitation. *E. globulus* was more likely to be present in areas with low temperature seasonality and high annual precipitation. Thus, considering future climatic changes for the Mediterranean basin, the natural recruitment (seedlings survival) of *E. globulus* will likely become harder. On the other hand, it was not clear how *P. pinaster* recruitment is affected by climatic conditions, indicating a high ecological plasticity of this species. However, most of the variability in these species establishment occurs at small spatial scales. Small-scale factors (vegetation cover, disturbance and habitat type) were more important than other high-scale factors (Climate and plantation characteristics) and they are essential to precise predictions at more local scales. Results from our survey revealed the suitability of all habitat types studied for *P. pinaster* natural regeneration, although their recruitment was more limited in natural forest. Forest and grassland were the most resistant habitats to *E. globulus* natural establishment while highly disturbed areas can be considered hubs for eucalypt density. Thus, monitoring and management efforts should focus on those sites with higher establishment probability (i.e. open areas) trying to promote native vegetation and reduce disturbance levels.

It´s fair to emphasize that the intensive management of these plantations (mainly *E. globulus*) might contribute to diminish their ability to develop a significant propagule pool. However, mature plantations (left unharvested) required an increased attention, as the propagule pressure builds with time.

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AGRADECIMENTOS

Depois de concluído este grande desafio não poderia deixar de expressar o meu profundo e sincero agradecimento a todas as pessoas que de alguma forma contribuíram para esta tese, em especial:

Às minhas orientadoras, Professora Otília Correia e Professora Cristina Máguas, pela supervisão desta tese. Obrigada por me acompanharem ao longo destes anos, por todo o apoio, amizade e orientação não só neste trabalho mas em todo o meu percurso científico.

Ao coordenador empresarial deste projeto, Engenheiro João Soares, por todo o apoio ao longo destes últimos anos, por ter proporcionado todas as condições que permitiram o desenvolvimento deste trabalho, por todos os conselhos e amizade.

Ao Centro de Ecologia, Evolução e Alterações Ambientais (cE3c), Faculdade de Ciências da Universidade de Lisboa (FCUL) e Fundação para a Ciência e Tecnologia (FCT), por terem apoiado e proporcionado as condições necessárias ao desenvolvimento desta tese.

À empresa The Navigator Company por ter acolhido este projecto, por proporcionar todo o apoio logístico e disponibilização de dados essenciais para a qualidade e sucesso deste estudo, por me terem recebido tão bem. Em especial o meu profundo agradecimento ao Engº. Gil Mata, ao Engº. João Lé, ao Engº. André Carvalho, à Engª. Paula Guimarães, ao Engº.Tiago Oliveira, e ao Engº. João Bandeira, por todo o apoio e acompanhamento deste trabalho. Não poderia também deixar de agradecer ao José Manuel Santos, à Irene Pinto e à Isabel Pereira por todo o apoio e amizade.

A todos os técnicos florestais da The Navigator Company que me acompanharam no trabalho de campo. Em particular ao Luís Alarico, Américo Campos, Francisco José Inácio, Isidro Costa, João Pires, Joaquim Fonseca, Jorge Lourenço, José Carlos Pereira, Leonel Almeida e Ricardo Mendes o meu profundo agradecimento pelo apoio essencial no trabalho de campo desta tese, por toda a partilha de conhecimentos e amizade.

A toda a equipa do Raiz. À Drª. Cristina Marques, Drº. Carlos Valente, Engº. Alexandre e Drª. Sofia Corticeiro, pelo apoio e partilha de conhecimentos.

Às minhas colegas e amigas da Florestal Ana Barros, Ana Marques, Inês Mirra e Sofia Soares pela vossa amizade e companhia.

To Pablo, thank you for all help with the macroecological models and statistical analysis, and all the knowledge that you provided me about R “world”. Thank you also for receiving me so welcoming in Seville.

Ao Pedro Pinho, Cristina Antunes e Alice Nunes por todos os conselhos no tratamento dos dados e análise estatística.

À Andreia Anjos, Márcia Vieira, Elsa Gonçalves e Sara Coelho pelos dias de trabalho de Campo.

Ao pessoal de vegetal (e não só), Alice N., Andreia A., Artur S. Cristina A., Cristina B., Cristina C., Florian, Helena S., Paula M., Pedro P., Ricardo C, Sérgio C., Silvana M., Teresa D. e Teresa M. pela vossa amizade e por tornarem todos os momentos de trabalho e lazer muito mais agradáveis.

Aos meus pais, por todo o apoio, pela segurança de saber que estão sempre ao meu lado, pela liberdade e pelos valores que incutiram em mim. À minha irmã Vera, por todo o apoio e amor (e claro pelo serviço de "babysitting").

À minha filha, Diana, por me ensinares tanto, pelo teu amor tão puro e por, mesmo sem saberes, seres a minha maior fonte de motivação e força.

A ti, João, a pessoa mais difícil de agradecer pois não existem palavras suficientes para demonstrar o que sinto. Obrigada por estares sempre ao meu lado e me apoiares incondicionalmente, mostrando sempre um grande interesse pelo meu trabalho. Pelas várias revisões aos meus textos. Pela partilha de todos os momentos que este estudo envolveu e pelo ânimo e confiança que construíste em mim e que foram essenciais para concluir este doutoramento.