Title

Management and fire, a critical combination for *Eucalyptus globulus* dispersal

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Abstract

In a context of growing demands for wood and wood derived products, plantations of exotic tree species have globally increased. Fast growth and high productivity made *Eucalyptus* one of the most successful tree genus around the world. Nevertheless, this genus is often associated with negative ecological impacts on biodiversity and ecosystem functioning and the risk of expansion is considered a major threat. *Eucalyptus globulus* is the most planted tree species in Portugal, but common silvicultural measures, including periodic control of the understory vegetation, have traditionally limited natural regeneration. However, forest fires constitute a main driver of *E. globulus* dispersal and regeneration and, under the current climatic change scenario, the possible extension of the summer fire regime to previous months in spring and/or later months in autumn, may have a profound effect on *E. globulus* dispersal capacity. Moreover, isolated eucalypt trees, seed-trees, are often left uncut and many plantations are poorly managed potentially increasing the risk of *E. globulus* dispersal. To evaluate the impact of both management and fire event dates on *E. globulus* dispersal, we assessed the establishment of saplings beyond plantations and seed-tree boundaries in absence of fire and after 2017 June and October fires in managed and unmanaged conditions. Sapling survival was also analyzed two years after fire. Our results point

out that sapling establishment in our study area is not a major concern in the absence of fire. Also, our findings showed that *E. globulus* establishment is highly dependent on the time of the year a fire occurs and that pre-fire management practices constrain *E. globulus* dispersal. We also found that seed-trees are high seed dispersers after fire even in managed conditions, deserving great concern. Additionally, high sapling survival two years after October fire indicate that out of season fires might constitute an emerging issue regarding *E. globulus* expansion.

Keywords: Sapling establishment; Eucalypt plantation; Seed-trees; Out of season fires.

1. Introduction

Globally, the use of exotic tree species in planted forests is a major component of terrestrial land use in temperate and tropical regions and has been increasing in order to respond to growing demands for wood and wood derived products (Dodet and Collet, 2012). Fast growth, high productivity, and product quality, ascribed to many exotic species, are the main reasons why they have been widely used (Turnbull, 1999). *Eucalyptus* is one of the most successful tree genus around the world. Being native from Australia it is nowadays rated the second most cultivated tree worldwide (Rejmánek and Richardson, 2013). Despite their economic and social benefits, exotic tree species such as eucalypts are often associated with negative ecological impacts on biodiversity and ecosystem functioning (Richardson, 1998; Hartley, 2002), including the risk of expansion to surrounding areas where they may outcompete native species (Richardson and Rejmánek, 2011).

In Portugal, *E. globulus* Labill. (Tasmanian blue gum), which was introduced in the middle of the 19th century (Alves *et al.*, 2007), occupies nowadays ca. 845.000 ha (ICNF, 2019) and is one of the main forest species in the country. Most *E. globulus* stands in Portugal have been planted intentionally and are managed for pulpwood production in rotation cycles of 10-12 years, usually followed by a second or third coppice rotation (Soares *et al.*, 2007). Recommended silvicultural measures include periodic control of the understory vegetation to reduce fire hazard and weed competition for water and nutrients. As a result, any putative regeneration of *E. globulus* is likely to be destroyed by vegetation control measures (Larcombe *et al*., 2013; Fernandes *et al*., 2016a). Nonetheless, isolated *E. globulus* trees are often left uncut and many plantations are poorly managed, resulting in the presence of old trees, often large and with high canopies with great reproductive capacity, potentially increasing the risk of *E. globulus* dispersal. In particular, these dispersed trees, named as seed-trees, are potential seed-sources (Adams *et al.*, 1994) whose presence may have critical implications for *E. globulus* natural regeneration dynamics.

Eucalyptus globulus mature seeds are held in the canopy, inside the capsules. They remain there for months or even a few years (Lamont *et al.*, 1991). Seeds will eventually be released and dispersed, as branches dry out and fall. This process is more frequent during autumn and winter (Calviño-Cancela and Rubido-Bará, 2013), or soon after a fire (Gill, 1997).

Given the wide range of environmental conditions and disturbances in which it has been established (Kirkpatrick, 1975) there have been concerns about *E. globulus* ability to regenerate and establish wildlings beyond plantations edges. These concerns resulted in several studies and Weed Risk Assessments have recommended *E. globulus* to be considered an invasive species in Europe, the Americas, New Zealand and the Pacific and Indian Ocean islands (e.g. Sanz-Elorza *et al.*, 2001; Rejmánek and Richardson, 2013; Gassó *et al.*, 2010; Gordon *et al.*, 2012; Marchante *et al.*, 2014). Nonetheless, other studies ranked *E. globulus* with moderate risk of invasion, including in well managed plantations (Larcombe *et al.*, 2013; Fernandes *et al.*, 2016a; Ziller *et al.*, 2018). In fact, Rejmánek and Richardson (2011) concluded that eucalypts have a low invasiveness potential compared with most other commercially important tree species, due to limited seed dispersal, high seedling mortality, and lack of compatible ectomycorrhizal fungi. Fernandes *et al.* (2016a) reported that *E. globulus* seedlings are not tolerant to drought stress, with high mortality immediately after emergence, despite the fact that once seedlings overcome the first two months, mortality rate decreases, as also suggested by Calviño-Cancela and Rubido-Bará (2013).

A key factor that impacts the risk of dispersal and regeneration are forest fires (Fernandes *et al.*, 2016b). This is particularly critical in fire prone regions of Mediterranean-type climate with long dry summers, such as Portugal (Salis *et al.*, 2014). Under the current climatic change scenario, the frequency of out of season fires is likely to increase (Turco *et al*., 2019), and may change the known pattern of this species expansion.

In Portugal, 2017 was a particularly dry and warm year, which resulted in several large wildfire events from June (early in the season) to mid-October (late in the season). As a consequence of the June fires ca. 52.000 hectares (ha) burned, while the October fires devastated an even larger area (ca. 190.000 ha) (ICNF, 2017). Fire events are a critical factor in stimulating *E. globulus* regeneration, through dorment bud's sprouting and seedling recruitment (Silva and Marchante, 2012; Larcombe *et al.*, 2013; Águas *et al.*, 2014, Calviño-Cancela *et al.*, 2018), but seasonal differences have not been studied in detail. High fire risk and a longer fire season raise concerns about more extensive naturalization events and an increase in potential invasive behaviors.

The present study looks at the effects of fire, including out of season events, and the compounding impact of forest management, along with the presence of seed-trees, on the success of *E. globulus* recruitment. In particular, we hypothesize that natural regeneration and potential expansion of *E. globulus* outside plantations and beyond the isolated trees (seed-trees) are affected by fire and management practices. However, we expect that high sapling mortality will limit *E. globulus* spread. In this context, the main objective of this study is to evaluate the impact of the 2017 June and October forest fires and previous plantation management on the natural regeneration and expansion capacity of *E. globulus* saplings. In order to achieve this, we evaluated the risk of *E. globulus* expansion by i) assessing the establishment of *E. globulus* saplings outside plantations edges and in the surrounding areas of isolated seed-trees in the absence of fire and after two distinct fire events, ii) analyzing the role of vegetation management actions in establishment outside plantation boundaries and area surrounding seed-trees, to ultimately mitigate the potential invasive behavior and finally iii) assessing the survival rate of saplings two years after fire occurrence. We expect this information will inform management decisions aimed at mitigating the risk of *E. globulus* invasion.

2. Material and Methods

2.1 *Study area*

This study was carried out in Northern and Central Portugal, regions of higher productivity for *E. globulus* (Alves *et al*., 2007), in sites affected by the fire events that occurred on 17th June 2017 (Castanheira de Pêra (CDP) and Pedrógão Grande (PG)) and on 15th October 2017 (Castelo de Paiva (CSP), Mira (M), São Pedro de Alva (SPA) and Pampilhosa da Serra (PS)) (Fig. 1a). Climate in the study area is temperate with dry and mild summer (Csb), according to Köppen climate classification (IPMA, 2021), with mean annual temperatures ranging from 10.5 to 14.6 °C and total precipitation ranging from 952 to 1440 mm (IPMA, 2021). Elevation varies from 15 to 735 m. This study area experienced a very dry winter and spring in 2017 (Fig. 1e and f). Site characterization is included in Supplementary Table S1.

Within the area affected by the June fire, three managed and three unmanaged *E. globulus* plantations were selected. In the October fire-affected areas, seven managed and five unmanaged *E. globulus* plantations were studied. Fourteen and 28 isolated seed-trees, affected by the June and the October fires, respectively, and 42 unburnt isolated seed-trees were also studied. Simultaneously, 18 unburnt unmanaged plantations located close to the selected burnt plantations were sampled. Moreover, data from a previous work with unburnt managed plantations (Fernandes *et al*., 2016a) was included in this study, where the same sampling design was applied and selecting only data from the same climatic region. After fire, plantations were classified as *managed* when no large trunks of shrubs and other tree species were observed (suggesting that an effective understory vegetation control was carried out) and in the case of coppice stands when the number of stems *per* stump were less or equal to three (which would indicate that appropriate thinning of the stand was carried out). In order to classify the type of stand management around seed-trees, surrounding areas were considered. Likewise, these areas were considered as *managed* if no large trunks of burnt trees or shrubs in nearby forest or shrublands were observed.

Sampling procedures required meeting several stringent criteria that highly restricted plantation and seed-tree selection. First, in order to ensure that the *E. globulus* saplings recorded belonged to individuals from the studied plantation, plantations were selected (i) without any *E. globulus* individuals in their adjacent areas; (ii) located at least 70 m apart from another *E. globulus* plantation and (iii) with no seed-trees nearby. The presence of seed capsules on the *E. globulus* trees was verified, to ensure the potential for natural regeneration. The size of the plantations, with preference for larger areas, and good accessibility were additional conditions for plantation selection. Finally, sampling for seed-trees implied the selection of trees (i) isolated from other seed-trees at least 70 m; (ii) with at least 35 cm of DBH (Diameter at Breast Height) and; (iii) with a completely burnt canopy.

Both plantations and seed-trees were classified according to *Type* (plantations or seed-trees), *Fire occurrence* (No fire and June or October fire) and *Management* (managed or unmanaged) (Fig. 1 b, c and d; Tab. 1).

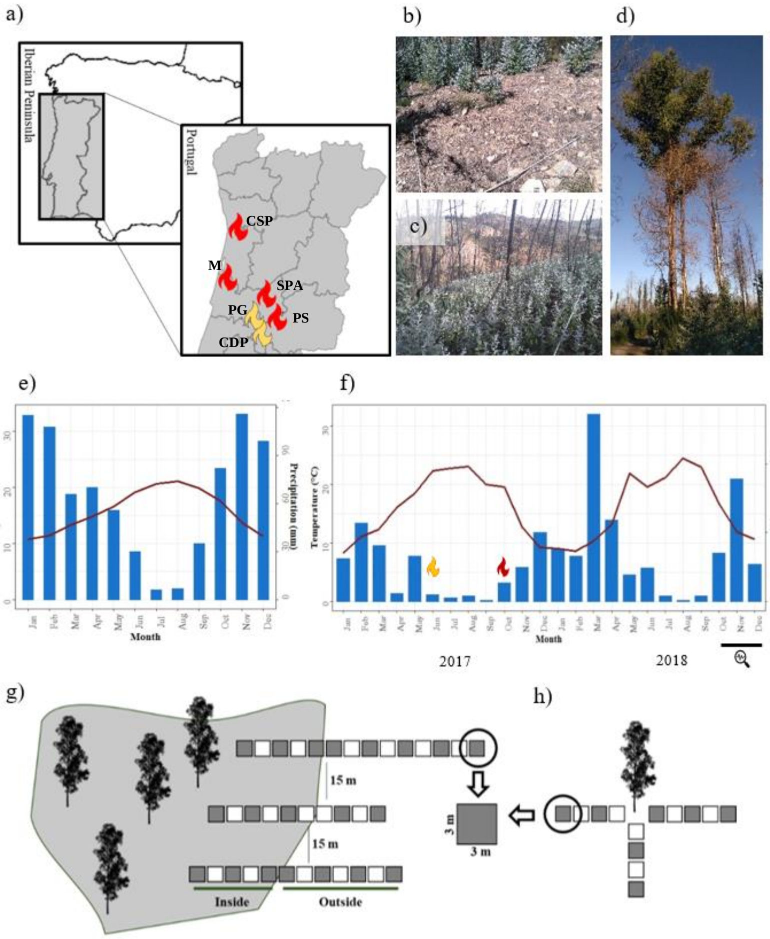

Figure 1: Location of the study regions in Portugal: a) study sites) affected by June fire are highlighted in yellow (Castanheira de Pêra (CDP) and Pedrógão Grande (PG)), while study sites affected by October fire are highlighted in red (Castelo de Paiva (CSP), Mira (M), São Pedro de Alva (SPA) and Pampilhosa da Serra (PS)); b) burnt managed *E. globulus* plantation; c) burnt unmanaged *E. globulus* plantation; d) burnt *E. globulus* seed-tree; e) monthly average temperature [ºC] (line) and monthly average precipitation [mm] (bars) in study areas from 1982 to 2012 (Climate-date.org) and f) from January 2017 to December 2018, with fire events and October to December 2018 data collection highlighted. Schematic diagrams of transects design in plantation (g) and seed-trees (h): transects were established with 3×3 m plots. Only grey plots were sampled. Transects were oriented perpendicular to the plantation boundary edge or to the seed-tree.

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2.2 *Field sampling*

2.2.1 Assessing *E. globulus* dispersal and natural regeneration

Sampling was performed from September to December 2018, about one year after the fires. Data were collected through transects from *E. globulus* plantation edge to adjacent areas occupied by other habitats (Fig. 1 g). In each plantation, at least three transects (plantation transects) were established 15 m apart, perpendicular to the plantation's boundary edge, and each transect was divided in 3x3 m plots. The first plot was established at 0 or 3 m outside the plantation and then plots were sampled alternately (adapted from Callaham *et al.,* 2013). Number and cover of *E. globulus* saplings were quantified in each sampled plot. *Eucalyptus globulus* saplings were distinguished from coppice or planted individuals, through its position and size relatively to the planted trees (unkempt plantation lines) that were mostly already resprouting. Transect sampling finished when two consecutive plots had no *E. globulus* saplings, marking the limit of the *E. globulus* expansion. Within the plantation, three alternate plots were always sampled (starting at the symmetrical distance of the outside transect, *i.e*., if the first outside plot was sampled at 3 m, the same occurs to the inside plot, being marked as -3 m) (Fig. 1 g). Similarly, to assess *E. globulus* natural regeneration around seed-trees, at least two perpendicular transects (seed-tree transects) were sampled in each seed-tree with a maximum of four transects *per* tree (Fig. 1 h). In what concerns seed-trees, management type was classified at transect level, mostly due to location near roads and/or adjacent habitats. Sampling finished when two consecutive plots had no *E. globulus* saplings. This means that, in both cases, there were always at least two plots to the outside of the plantation and in the surrounding areas of seed-trees. The total number of transects sampled is summarized in Table 1.

Table 1: Total number of transects sampled within the study area, including plantation and seed-tree transects, both managed and unmanaged and organized by Fire Occurrence (No Fire, June Fire and October Fire).

	No fire		June Fire		October Fire	
	Managed	**Unmanaged**	**Managed**	**Unmanaged**	**Managed**	**Unmanaged**
Plantation	277	54	10	13	30	21
Seed-tree	56	86	27	20	39	45

Due to the high density of saplings along the seed-tree transects and the difficulty to count all of them, the possibility of using sapling cover instead was evaluated. The relationship between the total number and the area covered by the *E. globulus* saplings was examined through a Spearman correlation (Supplementary Figure S2) and, as expected, Spearman r value (r=0.88) confirmed that both parameters were highly correlated and consequently we proceeded to use sapling cover to characterize the incidence of natural regeneration.

2.2.2 Assessing *E. globulus* sapling survival

Considering only *E. globulus* burnt plantations, permanent plots with 1 m radius were set, in which *E. globulus* sapling number was assessed. These permanent plots were included inside 3x3 m plots sampled. To evaluate sapling survival, these permanent plots were also monitored in October 2019, about two years after the fires, and the remaining saplings were recounted.

2.3 *Data analysis*

Eucalyptus globulus dispersal curves focused on fire events were constructed by plotting sapling mean cover for each distance from inside and outside the plantation edge and from each seed-tree. Then, a multivariate local polynomial regression estimator (Loess) fitting was applied. Loess is a non-parametric method that fits a quadratic surface by weighted least squares (Cleveland and Devlin, 1988). To assess the main factors affecting dispersal distances in *E. globulus* after fire, we used a Generalized Linear Mixed Model (GLMM) with a negative binomial distribution to account for unbalanced design and overdispersion associated with the high number of zeros in the data. *Fire occurrence* (only "June fire" or "October fire"), *Management* and *Type* were included in the model as fixed factors and the variable *Site* as random effect (Tab. 2). Maximum distance reached by saplings was defined as the dependent variable.

Similarly, to assess the factors that influenced *E. globulus* natural regeneration, sapling cover was also modelled performing a GLMM with a negative binomial distribution and using *Fire occurrence*, *Management* and *Type* as fixed factors and *Site* as random factor. In order to guarantee the same number of sampled plots in each transect (i.e. two plots, see Field sampling), only the plots located within the first 15 m outside of the plantations and around seed-trees were considered. Finally, to determine which groups were significantly different, multiple pairwise comparisons of estimated marginal means were calculated, using the *emmeans* function. Bonferroni correction method was applied to adjust p values to multiple comparisons.

Sapling survival was evaluated through the comparison between the number of saplings counted one year (2018) and two years (2019) after June and October fires, in sampled plantations using Wilcoxon tests.

Data analysis was performed using packages *stats*, *glmmADMB* and *emmeans* in R studio software (v.3.6.1).

Table 2: Description of explanatory variables.

Variable	Data description
Type	Plantation or isolated seed-tree
Fire occurrence	No fire, June or October fire event
Management	Managed - trunks *per* tree were less or equal to three and if large trunks of burnt shrubs or trees were not observed Unmanaged - trunks *per* tree were higher than three and if large trunks of burnt shrubs or trees were observed

3. Results

3.1 Fire date and management effects in **E. globulus** *dispersal*

Sapling cover and distance to both plantation edges and seed-trees showed a clear space-dependent relationship. The fit between sapling cover and distance to plantation edge indicated that, although there were no statistically significant differences (data not shown), highest cover of saplings always occurred inside plantations (from -15 to 0 m), decreasing to the outside. This pattern was also observed along the isolated seed-tree transects, with the maximum cover of *E. globulus* saplings observed close to the seed-trees (from 0 to 15 m) and the cover values decreasing with increasing distance from these trees.

GLMM results indicated that Seed-tree, October fire and Unmanaged condition are significantly increasing the distance at which *E. globulus* saplings occur (Tab.3). For both plantations and seed-tree transects sapling cover was significantly higher after the October fire than that observed after the June fire (Fig. 2).

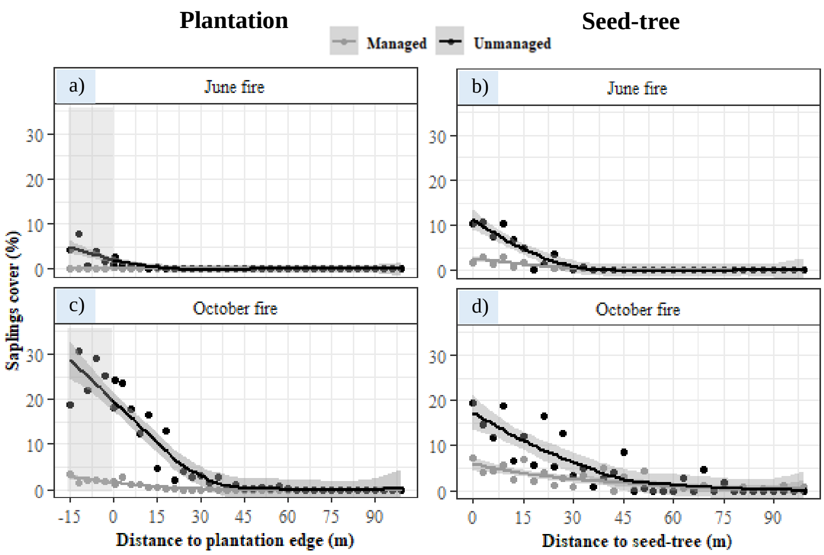

Figure 2: Dispersal curves of *E. globulus* saplings after the June and October 2017 fires, based on average sapling cover (%) to the edge of managed and unmanaged *E. globulus* plantation (a and c) and managed and unmanaged surrounding areas of *E. globulus* seed-trees (b and d) using Local Polynomial Regression

Fitting (Loess). Shaded area represents 95% confidence intervals. Negative distances (highlighted in grey) represent the inside of *E. globulus* plantations. The distance of 0 m represents plantation edge and seed-tree location.

When comparing unmanaged and managed conditions, in addition to a greater sapling cover, in the first, distances reached by *E. globulus* saplings were higher in unmanaged conditions for both plantations and seed-trees. Also, after the October fire, saplings were found at greater distances (45 and 75 m in plantation and seed-tree transects, respectively), while in plantations and seed-trees burnt in June, saplings reached 15 and 30 m, respectively. Saplings were observed to a maximum distance of 99 m away from seed-trees and 69 m from unmanaged plantations edge after the October fire, but at very low frequencies (with only one sapling observed in each case).

Table 3: Generalized linear mixed model (GLMM) using *Type* (Plantation or Isolated Seed-tree), *Fire date* (June fire and October fire) and *Management* (Managed or Unmanaged) to model maximum distance reached by *E. globulus* saplings. Coefficients of the model, standard errors, the z statistic and the associated probabilities: **: ≤ 0.01; *: < 0.05.

	Estimate	**Std. Error**	**z value**	**Pr(> |z|)**
(Intercept)	-2.306	1.167	-1.98	**0.048 ***
October fire	2.687	1.237	2.17	**0.029 ***
Seed tree	3.259	1.151	2.83	**0.005 ****
Unmanaged	2.626	1.209	2.17	**0.029 ***
October fire * Seed tree	-2.139	1.223	-1.75	0.080 .
October fire *Unmanaged	-0.091	1.311	-0.07	0.945
Seed tree * Unmanaged	-1.316	1.293	-1.02	0.309
October fire * Seed tree * Unmanaged	-0.169	1.436	-0.12	0.906

3.2 E. globulus *sapling establishment and survival*

Considering *E. globulus* establishment, GLMM results showed that all studied factors were influencing sapling cover in our study area (Tab. 4).

Table 4: Generalized linear mixed model (GLMM) using *Type* (Plantation or Isolated Seed-trees), *Fire occurrence* (No fire, June fire and October fire) and *Management* (Managed or Unmanaged) to model *E. globulus* sapling cover. Coefficients of the model, standard errors, the z statistic and the associated probabilities: **: ≤0.01; *: < 0.05.

	Estimate	**Std. Error**	**z value**	**Pr(> |z|)**
(Intercept)	-2.490	1.229	-2.03	**0.043 ***
No fire	2.226	1.223	1.82	0.069 .
October fire	3.072	1.296	2.37	**0.018 ***
Seed tree	3.337	1.283	2.60	**0.009 ****
Unmanaged	2.742	1.369	2.00	**0.045 ***
No fire * Seed tree	-5.565	1.412	-3.94	**$8.1e^{-5}$ ****
October fire * Seed tree	-2.183	1.393	-1.57	0.117
No fire * Unmanaged	-6.466	1.725	-3.75	**$1.8e^{-4}$ ****
October fire *Unmanaged	-0.176	1.519	-0.12	0.908
Seed tree * Unmanaged	-1.452	1.510	-0.96	0.336
No fire * Seed tree * Unmanaged	4.947	1.988	2.49	**0.013 ***
October fire * Seed tree * Unmanaged	-0.122	1.711	-0.07	0.943

The absence of fire revealed the lowest sapling cover (around 0%) when compared to the occurrence of fire, both in plantations and seed-trees, with no significant statistical differences observed also between managed and unmanaged regimes (Fig. 3).

After the June fire, the highest mean sapling cover was observed under unmanaged seed-trees (9.2±2.5%), while managed and unmanaged plantations and managed seed-trees showed low sapling cover (0.1±0.1%, 1.4±0.8% and 2.4±0.7%, respectively) with no statistically significant differences. On the other hand, the October fire showed heterogeneous results, with low sapling cover in managed plantations (1.5±0.5%), followed by managed adjacent seed-trees areas (6.5±1.8%) while sapling cover had the highest value in both unmanaged plantations and unmanaged adjacent areas to seed-trees (19.4±4.4% and 13.8±2.8%, respectively) (Fig. 3). Comparing *E. globulus* sapling cover from plantations and seed-trees, higher sapling cover under seed-trees was always observed, except when comparing with unmanaged plantations, after the October fire.

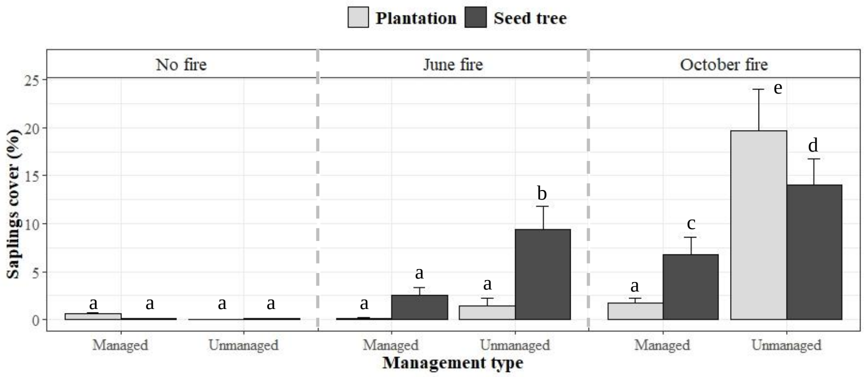

Figure 3: *Eucalyptus globulus* sapling cover (%; mean ± SE) for managed and unmanaged *E. globulus* plantations and managed and unmanaged surrounding areas of seed-trees in absence of fire (No Fire) and after June and October 2017 fires. Letters indicate significant differences based on multiple pairwise comparisons of estimated marginal means with Bonferroni adjustment.

Furthermore, Wilcoxon tests showed statistically significant differences between the number of *E. globulus* sapling counted in 2018 and recounted in 2019, except in managed plantations affected by the June fire (Fig. 4). In managed plantations affected by the June fire, sapling number remained the same (128 saplings/ha) while in unmanaged plantations, a decrease was observed (2008 to 175 saplings/ha). Managed and unmanaged plantations burnt in October followed the same trend (a decreased from 3255 to 1498 and from 25413 to 19414 saplings/ha, respectively, from 2018 to 2019). It is important to highlight that, despite the low number of saplings, survival was higher in managed plantations after the June fire (100% of survival). Notwithstanding, unmanaged plantations affected by the June fire revealed 25% of sapling survival. After the October fire, both managed and unmanaged plantations showed almost 75% of sapling survival (data not shown).

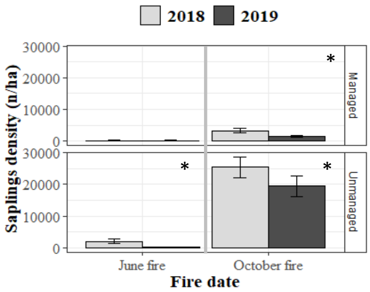

Figure 4: Wilcoxon tests comparing *E. globulus* sapling density (n/ha; mean ±SE) assessed in 2018 and 2019 for managed and unmanaged *E. globulus* plantations affected by the June and October 2017 fires. Asterisks represent significant differences (* p<0.05, ** p<0.01).

4. Discussion

Our study found that the time of the fire event (naturally associated with after-fire weather conditions) is determinant for the success of *E. globulus* natural regeneration. In addition, management before fire is crucial to reduce the dispersal and degree of sapling cover.

It was also observed that the existence of seed-trees greatly contributes to *E. globulus* expansion. Moreover, our results indicate that spring and autumn fire events have an additional impact on sapling establishment. After the June fire, sapling cover was always lower than that found after the October fire. Nevertheless, differences were only significant for unmanaged plantations, underlying the importance of the synergistic effects between these factors. Most seedlings germination occurred immediately after fire (Pryor, 1976), when bare soil and favorable weather conditions were available, which is more likely to occur after an autumn fire (October fire) which usually precedes wet weather, than after a spring fire (June fire) which is usually followed by dry summer conditions.

Previous studies with spring sowing registered high mortality of *E. globulus* seedlings, associated with high temperatures and drought (Fernandes *et al.*, 2017). Furthermore, dos Santos *et al.* (2015) showed that seed release from capsules held in scorched branches could last eight weeks and Nereu *et al.* (2019) suggested that ca. 70% of seedlings germinated within the first month after sowing. Taking into consideration this information and the fact that 2017 summer was extremely hot and dry, we suggest that the low sapling cover found after the June fire could be associated with high mortality due to dry conditions. Consequently, saplings found after the June fire were therefore either able to survive the summer drought or germinated in the rainy season as suggested by Silva *et al.*, 2021, which mentions recruitment as a *continuum*. Thus, regardless of the existence of management actions, the June fire has low *E. globulus* natural regeneration.

On the other hand, unmanaged areas surrounding seed-trees had a higher sapling cover. The absence of management and clearing actions lead to a conspicuous capsule accumulation on the soil (ca. up to 10 cm depth, field observations). During a fire only the capsules located in the upper soil layer burn, while the ones of the lower layers remain intact, protecting seeds from the heat damage (dos Santos *et al.*, 2015), which may enable later germination. Additionally, sprouting from seed-trees up in the canopy (Fig. 1d) may provide moderate shade and decrease high soil temperatures, creating more favorable conditions that facilitate seedling recruitment and survival, contributing to the higher sapling cover observed.

Conversely, after the October fire, due to the subsequent rainy season, weather conditions were favorable to seedling establishment, resulting in high sapling cover one year after fire. However, managed plantations showed low sapling establishment. It is known that *E. globulus* reaches sexual maturity at three to four years (Jordan *et al.*, 1999), but in dense plantations seed production is suppressed and does not usually occur until trees are at least seven years old (Kirkpatrick, 1975). In fact, rotation cycles of 10-12 years reduce seed accumulation because trees are harvested before reaching their full potential of seed production, which could explain the low sapling cover within managed plantations. Also, capsules accumulation in the canopy is lower than in unmanaged plantations, where trees are left uncut for many years, accumulating capsules produced in different years, increasing their reproductive capacity (Barbour *et al.*, 2008). Additionally, studies are needed to compare capsules accumulation in the canopy and in the soil as well as seed accounting and viability in trees with different ages, including seed-trees.

Similarly to other studies performed in Portugal (Fernandes *et al.*, 2016a; Águas *et al.*, 2017), we confirmed that in the absence of fire, *E. globulus* sapling cover in surrounding areas to both *E.*

globulus plantations and seed-trees is extremely low. *Eucalyptus* seeds require wet and bare soil to germinate (Rejmánek and Richardson, 2011) and in the absence of fire, areas outside plantations are dominated by vegetation that compete (Garau *et al.*, 2009) and shade the soil surface. Additionally, the accumulation of litter on the soil hinders *E. globulus* seedlings emergence (Mount, 1964; Águas *et al.*, 2017). Also, litter prevents capsules and seeds from falling directly on the soil surface, decreasing the emergence rate (Calviño-Cancela *et al.*, 2018). These effects, combined with the fact that seed recruitment is positively related to disturbance (Fernandes *et al*., 2018), may explain the lack of *E. globulus* germination in the absence of fire and the increase of sapling cover found in plantations affected by fire. After a fire, seeds have favorable environmental conditions for their establishment, namely greater light and nutrient availability as a result of the combustion of vegetation and litter (Calviño-Cancela *et al.*, 2018).

In this study, seed-trees were considered as a key propagule source, due to their age and seed production potential. Isolated seed-trees showed higher sapling cover, except when comparing with unmanaged plantations affected by the October fire. This may be due to a much higher capsule production capacity (field observations) and lack of competition from coppice or sprouting from pre-existing trees in plantations (Potts, 1986). Higher sapling cover in unmanaged plantations after the October fire compared to seed-trees could be due to the higher seed production ascribed to the higher number of trees present in plantations.

Regarding sapling establishment distances, for all the studied transects, a clear decrease was observed from both *E. globulus* plantations edges and *E. globulus* seed-trees, until around 45 m. Maximum saplings distances were 69 m away from plantations (similar to Deus *et al.*, 2019) and 99 m from seed-trees, both observed in unmanaged situations after the October fire. Greatest distances reached by saplings from seed-trees are probably due to the higher height of these trees (ca. 17 m comparing with 10 m in plantations), since it is known that *E. globulus* seeds do not have an effective wind dispersal mechanism (Kirkpatrick, 1977). Expected distances are reported to follow approximately twice the tree's height (Cremer, 1977). Moreover, the greatest cover of saplings was always found within the plantations and along the first 15 m outside the plantations, corresponding largely with results observed by other authors (Calviño-Cancela and Rubido-Bará, 2013; Larcombe *et al*., 2013; Fernandes *et al*., 2016a; Águas *et al*., 2017; Deus *et al*., 2019), reflecting the limited seed dispersal ability of *E. globulus*. Further studies could be performed through geographic modelling of *E. globulus* expansion, to better understand its behavior and dispersal risk, as suggested in the Global Guidelines concerning non-native trees use (Brundu *et al*., 2020).

Ashton and Chinner (1999) described that there is an antagonist set of conditions between germination and establishment, since for seedlings emergence bare soil is crucial, while sapling establishment requires shadowed conditions, associated with water availability. Hence, saplings assessed one year after fire overcame a high mortality period (Calviño-Cancela and Rubido-Bará, 2013) and found favorable conditions for establishment. It is important to add that, one-third of *E. globulus* plantations were managed after fire and some two years saplings were eliminated by those practices, as reported by Águas *et al*., 2014. Moreover, it was observed a high mortality in permanent plots, maybe due to interspecific competition like herbaceous and shrubs (Fernandes *et al*., 2018; Deus *et al*., 2019) and also because one-year old saplings are more sensitive (Garau *et al*., 2009). Most of the saplings recounted two years after fire, were more than 1.5 m in height and, as suggested by Adams *et al*. (2003), were able to overcome potential competition from other species. However, at high *E. globulus* sapling densities reduction in sapling number is expected, as a consequence of the increasing intra-specific competition.

Other studies performed in burnt areas in Portugal reported 8.800 *E. globulus* plants/ha five years after fire in abandoned plantations (Silva and Marchante, 2012) and 4.800 plants/ha seven years after fire (Águas *et al.*, 2014). Nevertheless, these studies are likely to be site and year dependent. We found that unmanaged plantations burnt in October 2017 showed a significantly higher sapling density (ca. 20.000 saplings/ha, two years after fire). This data represents the worst-case scenario: an autumn fire, followed by rain and combined with the absence of plantation management. The synergetic effect observed between fire and poor management highlights the importance of forest management practices in the control of natural regeneration and expansion of *E. globulus*. Along with *E. globulus* plantations, we emphasize the importance of isolated seed-trees across the Portuguese landscape in the establishment and expansion of this species. We underline the importance of considering it as an additional factor when planning control actions to prevent *E. globulus* expansion beyond plantations.

Conclusions

This study clarified the conditions in which *E. globulus* could represent a risk of significant dispersal to areas surrounding plantations or isolated seed-trees after a fire. Our findings indicate that *E. globulus* establishment is highly dependent on both the time of the year a fire occurs and the management practices implemented prior to fire. In the absence of fire, even in unmanaged plantations, sapling establishment does not seem to be a major concern in our study area (mainly Central Portugal), especially when fires occur early in the season followed by several dry months during summer. In this context, adequate management seems to be an essential measure to prevent *E. globulus* natural dispersal inside or around the borders of plantations once regular harvesting will decrease capsule production and its accumulation, associated with younger age of trees. Furthermore, even when an autumn fire events occur, just before the rainy season, *E. globulus* sapling establishment in well managed plantations is very low. On the other hand, we found a significant increase in sapling cover in both unmanaged plantations and isolated seed-trees after the October fire. Therefore, it is critical to consider that, under a warmer weather, changes in frequency, intensity and seasonality of fires are expected, influencing *E. globulus* dispersal behavior. In this context, adequate management seems to be an essential measure to prevent *E. globulus* establishment inside or around the borders of plantations. Furthermore, we found that isolated seed-trees seem to be an overall effective seed disperser after the advent of a fire, regardless of previous management actions.

Future studies should assess whether other specific local variables along with out of season fires occurrence could further influence sapling establishment and survival. Once it is known that these events are becoming more frequent it is crucial to fully understand the potential invasive behavior of the species, especially considering autumn fires that are followed by rainy season. By increasing our knowledge about *E. globulus* expansion risks, we will contribute to improve best practices in plantation management of this species in Portugal.

Author contributions

AA conducted fieldwork. All authors contribute to the conceptualization and methodology design, AA and SC analyzed the data. AA led the writing and all the authors made substantial contributions to the writing. All authors have read and agreed to the published version of the manuscript.

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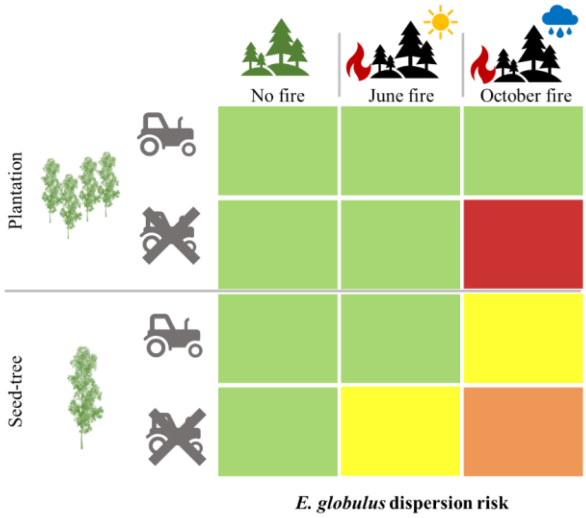
Graphical abstract



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Author statement

AA conducted fieldwork. All authors contribute to the conceptualization and methodology design, AA and SC analyzed the data. AA led the writing and all the authors made substantial contributions to the writing. All authors have read and agreed to the published version of the manuscript.