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SHORT COMMUNICATION

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Maritime pine natural regeneration in Coastal Central Portugal: Effects of the understory composition

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Abstract

Aim of study: To study the natural dynamics of *Pinus pinaster* natural regeneration in a post-fire scenario in three populations of Coastal Central Portugal, with severe ecological degradation due to plant invasions, and partially affected by the 2017 wildfires.

Area of study: Three *P. pinaster* populations, located along a geographic gradient of about 140 km on Coastal Central Portugal: Mira, Tocha and Leira.

Materials and methods: The density and establishment success of *P. pinaster* regeneration was studied in burnt and unburnt forest stands. Special focus was given to the effects of the cover by native and invasive species (mainly *Acacia longifolia*) on pine regeneration.

Main results: Regeneration values considered sustainable (over 0.3 seedlings·m^{-2}) were recorded in all three populations in burnt and unburnt plots, where only one of the three study sites showed signs of establishment difficulty. We recorded high levels of invasion which showed a negative relationship with regeneration density..

Research highlights: The cover of invasive flora was negatively correlated with pine regeneration, while the presence of native flora showed positive relations with density and height of pine natural regeneration.

Additional key words: *Pinus pinaster*; fire; competition; shrubs; invasive species..

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Introduction

Pinus pinaster forests occupy a significant part of the western Mediterranean basin (1.5 million hectares), covering the Atlantic meso-Mediterranean part of Portugal, also being distributed in Spain, France, Italy, Morocco, and Tunisia (Vinas *et al.*, 2016). Due to its great genetic variability, *P. pinaster* is very ecologically adaptable, being distributed along a wide range of climatic and edaphic conditions (Fernandes & Rigolot, 2007).

Fire is a natural shaper of Mediterranean ecosystems dynamics having a great influence on the composition,

evolution, and biological productivity of forest ecosystems. *P. pinaster* forests are amongst the most affected by recurrent wildfires in the Mediterranean Basin (Madrigal *et al.*, 2010). *P. pinaster* is an obligate seeder that often responds to fire through a quick dispersion of seeds, that can start during the fire and continue for a few months after the disturbance (Taboada *et al.,* 2017; Cruz-Alonso *et al.,* 2019), in many populations due to the germination of canopy seed bank inside serotinous cones (Maia *et al.,* 2012). Serotiny is common among pine species in fire prone ecosystems, and is defined as the capacity to retain seeds in long-closed cones within the tree canopy, requiring an environmental stimulus such as a heat shock for cone opening and seed dispersion (Hernández-Serrano *et al.,* 2013). The percentage of serotiny is highly variable; it is reported to range between 2 to 82% in Iberian populations, the Portuguese coastal populations being the least serotinous (Tapias *et al.,* 2004).

However, while serotiny may be determinant to explain the availability of seeds in post-fire colonization, this is not necessarily the case when there is no fire occurrence, or when other types of environmental disturbance occur, such as wood extraction or clear cutting. The interaction between the regeneration potential (even low) and local conditions such as soil characteristics or plant cover is paramount; the success of the establishment strongly depends on the biotic and abiotic conditions at the site (Maia *et al.*, 2012).

The understory vegetation has a key role in the forest ecosystem, namely on water regulation processes (Balandier *et al.*, 2022) and effect on forest trees regeneration (Malik, 2003). Regeneration patterns can be different according to the composition of the understory that exists at the site; depending on the species, natural succession is affected through interspecific competition (De las Heras *et al.*, 2012). Competition is, therefore, differential, and more marked in understory dominated by native scrub and grasses (Calvo *et al.*, 2008). On the other hand, some studies show a facilitation effect with the understory, namely regarding seedlings height (De las Heras *et al.*, 2012). This interspecific facilitation can happen in the presence of nursing species, such as the Spanish heath (*Erica australis*), strawberry-tree (*Arbutus unedo*) and gorse (*Genista tridentata*), typical species of the understory of *P. pinaster* forests (Calvo *et al.*, 2008).

Pine forests in Coastal Central Portugal, are commonly prone to plant invasions, particularly, but not exclusively, by the woody *Acacia longifolia* and the herbaceous *Conyza* spp. and *Cortaderia selloana*, due to the proximity of highly affected habitats and land uses (dunes – *Acacia* and agricultural areas – *Conyza* and *Cortaderia*). The community changes imposed by the presence of these invasive plants are known to have impacts on plant-soil nutrient status (Sardans *et al.*, 2016), to profoundly change the community composition, leading to decreased biodiversity (González-Muñoz *et al.*, 2016) and expectedly, to have a negative impact in the potential for pine natural regeneration to produce exploitable natural forests. Under the expected scenario of increased aridity, these species are likely to expand, in part due to the superior root growth and colonization ability under drought (Peñuelas *et al.*, 2017), but also due to the indirect effect of projected climate changes in fire occurrence, that is known to result in invasive species proliferation (Reilly *et al.*, 2020).

The specific objectives are then, to 1) investigate the natural regeneration of maritime pine in three different populations: Mira, Tocha, and Leiria and 2) evaluate how the understory composition, in terms of native and exotic species, affects natural regeneration after fire and without fire occurrence. The overall aim of this work is to study the natural dynamics of *P. pinaster* natural regeneration in a post-fire scenario in three populations of Coastal Central Portugal, with severe ecological degradation due to plant invasions, and partially affected by the 2017 autumn fires. For this purpose, the natural regeneration of maritime pine, and the description of the relative abundance of other species were assessed along a latitudinal gradient, along three locations of Coastal Central Portugal (Mira, Tocha, Leiria).

Material and methods

Study area

Three *P. pinaster* populations, located along a geographic gradient of about 140 km on Coastal Central Portugal (Fig. 1) were selected to investigate the natural regeneration of *P. pinaster*: Mira with an area of 37 km^2, Tocha with 16 km^2, and Leiria with an area of 33 km^2. These populations, managed by the Portuguese Forest Institute (ICNF) as production forests, were strongly affected by the fires of October 2017. The region of all three populations belongs to the Mediterranean Csb climate zone, temperate climate with dry and mild summers, according to the Köppen classification (https://www.ipma.pt/en/oclima/normais.clima/).

Experimental design and sampling

Maritime pine populations, pine recruitment, vegetation cover and site characteristics were evaluated in unburnt mature stands, and in stands that suffered a stand replacing fire (hereafter burnt) during the wildfires of 2017. In the summer of 2019, a total of 18 sampling points was established along the three populations of study. Three sampling points *per* condition (burnt or unburnt) were established within each population (Mira, Tocha, Leiria). The location of the sampling points was selected among the maritime pine forest cover at each one of the three regions, established at a random stratified manner, so that only patches representative of the typical conditions were included; areas that showed evidence of abandonment (*e.g.*, severe

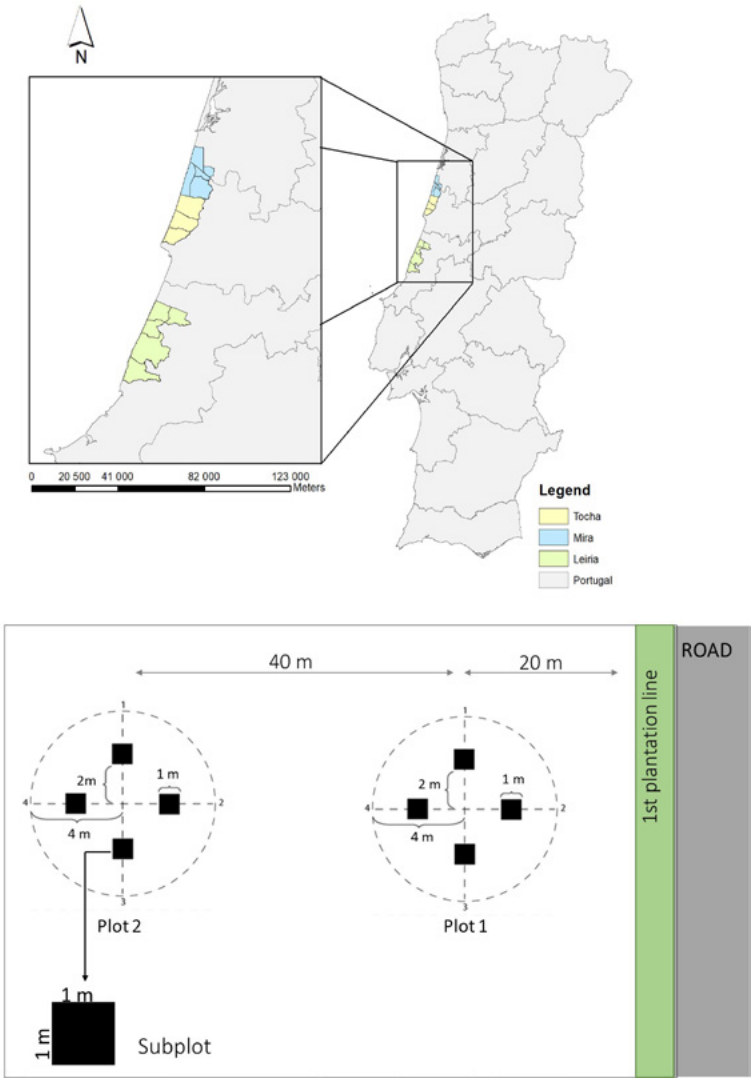

Figure 1. Selected *Pinus pinaster* populations Mira (M), Tocha (T) and Leiria (L) and schematic representation of the sampling design.

invasion by alien species, highly dense and underdeveloped pine trees due to lack of management) or that showed evidence of recent mechanical removal of the understory were not included (Table S1 [suppl]).

The experimental design was adapted from Maia *et al.* (2014); a sampling point was composed of two circular plots (plot 1 and plot 2), established on the forest stand according to the distance from the main access road. At each location, the first circular plot was established 20 m away from the first line of pine trees, and the second was installed 40 m away from the centre of the first plot. Each plot had a circular shape with 4 m radius, divided by two perpendicular lines crossed in the centre of the plot. Four micro-plots (1 m^2) were established at each plot, 2 m away from the centre of the plot, following the perpendicular lines, hence totalizing 8 micro-plots at each sampling point (Fig. 1). Field sampling followed a hierarchical strategy, with different data being sampled at the pine stand level, at the circular plots, or at the 1-m^2 micro-plot.

Maritime pine regeneration

Maritime pine natural regeneration density was evaluated at the micro-plot level, at each of the four 1-m^2 squares included inside each circular plot. The height (from the root neck to the last whorl) and age (estimation trough whorl counting) were also assessed for each live seedling.

Understory vegetation

The understory vegetation cover was evaluated at all plots, classified as native or invasive species, and further divided in woody or herbaceous (excluding the regenerated pine seedlings). For further simplification, the terms "native" and "invasive" will be used, since all the exotic plant species found in the study area are legally considered invasive (Diário da República, 2019). The following categories and sub-categories were considered: a) total native

Table 1. Spearman's correlations between regeneration response variables (density and height) and understory cover variables considered for the study.

Understory cover variables [1]	Pine natural regeneration [2]	
	Density ($n \cdot m^{-2}$)	Height (cm)
Unburnt sites		
Inv. herb. cover (%)	-	-
Inv. woody cover (%)	0.564 (ns)	-
Inv. total cover (%)	-	-
Total plant cover (%)	0.654*	-
Nat. height (cm)	-	0.485 (ns)
Total height (cm)	-	0.785** (+)
Burnt sites		
Inv. trees cover (%)	-0.419 (ns)	-
Nat. cover (%)	-	0.717** (+)
Nat. herb. cover (%)	-	-
Nat. woody cover (%)	-	0.799** (+)
Inv. height (cm)	0.619** (-)	-
Nat. height (cm)	0.562* (-)	-

[1] Inv: invasive. Herb.: herbaceous. Nat. Native. [2] –: no correlation. (-): negative correlation. (+): positive correlation. *, **, ns: p-values < 0.05, < 0.01 and non-significant, respectively.

plant cover, subdivided into woody and herbaceous; b) total invasive cover, also subdivided in woody and herbaceous. Cover was estimated as the visual ration of the projection of the area occupied by the individuals of each sub-category relatively to the area of the plot. The mean height of each category (native or invasive) was also estimated at the level of the circular plots.

Statistical analysis

One-way ANOVA, followed by Tukey test, was used to analyse differences in the age of seedlings, between conditions (burnt and unburnt) and/or populations. All the other variables were analysed by using the Kruskal-Wallis test, followed by the pairwise Wilcoxon test, as data did not meet ANOVA assumptions. Spearman's correlations were used to investigate possible relations between regeneration variables, and the vegetation variables: native total, woody and herbaceous cover; invasive total, woody and herbaceous cover; native height and invasive height. Correlations with $\rho > 0.35$ ($p < 0.05$) were considered due to the high variability of the field data. Data analysis was performed on R software, vers 4.1.1. using the packages *stats* (4.1.1), *ggpubr* (0.4.0), and *dplyr* (1.0.8).

Results and discussion

Location Mira had the highest pine regeneration density both in unburnt (6.6 seedlings$\cdot m^{-2}$) and burnt (1.5 seedlings$\cdot m^{-2}$) sampling points. For the three populations, the density of pine regeneration was greater in the unburnt plots, being this difference more pronounced in Mira. Leiria had the lowest value of pine regeneration in the unburnt condition (2.6 seedlings$\cdot m^{-2}$), and Tocha in the burnt condition (0.75 seedlings$\cdot m^{-2}$) (Fig. 1). There were no differences in the density of living pine seedlings between populations for both conditions, except in Tocha (Fig. 1).

In the burnt condition, Table 1 shows a positive relation between native cover and regeneration height, which could be explained by facilitation, through chemical/physical changes in the soil, or protection against herbivory (Gómez-Aparicio *et al.*, 2004, 2005). There were indications of positive relationship between regeneration height and native plant cover (ρ= 0.717; p<0.05), on the other hand, the negative relationship between the pine density and native cover height suggests a limit for this facilitation relation (Gómez-Aparicio *et al.*, 2005). Also in the burned condition, the regeneration density showed an overall negative relationship with the understory height, particularly with the invasive tree cover (Table 1), demonstrating a possible interspecific competition between the regenerating pines and the invasive cover, especially *Acacia longifolia*. This species causes major changes in the structure of the forest and negative correlation of its abundance with pine regeneration on coastal populations has been documented in the past (Rascher *et al.*, 2011).

In all the study sites, the regeneration density sampled in 2019 was adequate for production forests, since in all

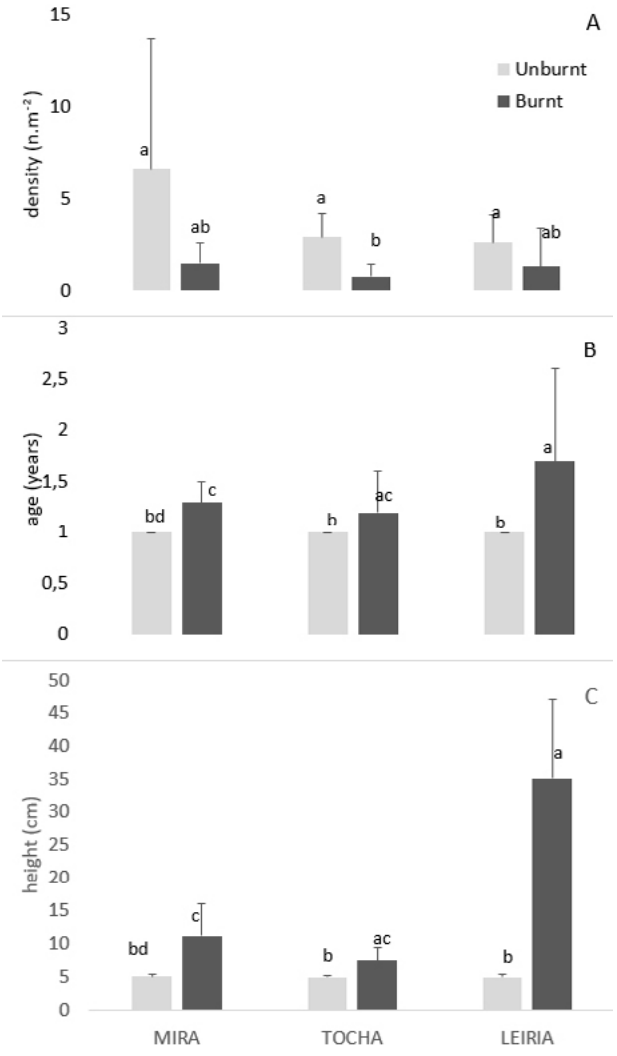

Figure 2. *Pinus pinaster* natural regeneration variables, for the three studied locations (Mira, Tocha and Leiria), and both conditions (light grey – unburnt, dark grey – burnt): A) mean seedling density (seedlings·m^{-2}), B) mean seedling age (years); C) mean seedling total height (cm). Error bars are standard deviations. Different letters indicate statistically significant differences (p<0.05) between each combination of populations × condition, for each of the studied variables.

cases the observed densities were higher than 3000 seedlings·ha^{-1}, or 0.3 seedlings·m^{-2} (Guignabert *et al.*, 2020) adapting forestry practices to improve seedling establishment is of prime importance to maintain sustainable forest management. In the coastal dune forests of maritime pine in SW France, regeneration failure after clear-cutting has greatly increased in the last decades. The aim of this study was to quantify the different stages involved in the regeneration process (seed rain, germination, survival. However, invasive plants in the area may be playing a role in the success of regeneration and seedling establishment. In Leiria, the lowest regeneration densities coincided with the higher seedling age range and height, presenting simultaneously the tallest (35.1 ± 12 cm) and the oldest (1.7 ± 0.9 years old) post-fire regenerated seedlings (Fig. 2). This relative success in seedling establishment after the first year, could be partially explained by the lowest level of invasive cover found in this population (Table S2 [suppl]).

Conclusions

This work provides insights into the contrasting interspecific relations between regenerating pine seedlings and the understory vegetation. Invasive species are suggested to be detrimental to pine natural regeneration and establishment. Early regeneration benefits from the presence of native vegetation. The pine stands with the most promising regeneration are in Leiria, that presented older and tallest seedlings, indicating a greater establishment success. Management measures are suggested, such as selectively controlling invasive species, both after and in the absence of fires, and preserving native vegetation, that could contribute to greater survival and establishment success, particularly in weakly serotinous populations, such as those in Coastal Central Portugal.

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Software: Not applicable.
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Visualization: P. Maia, S. Corticeiro, D. Rodrigues.
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