Universidade de Aveiro
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From seed to sapling - effects of plant community, soil cover and fire in the recruitment and establishment of maritime pine

Da semente à plântula– efeitos da comunidade vegetal, cobertura do solo e fogo no recrutamento e estabelecimento de pinheiro-bravo

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia Aplicada, realizada sob a orientação científica da Doutora Paula Alexandra Aquino Maia, Investigadora do Departamento de Biologia da Universidade de Aveiro e coorientação da Doutrora Sofia Caçoilo Corticeiro, Investigadora do Departamento de Biologia da Universidade de Aveiro

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“Between every two pines is a doorway to a new world” – John Muir.

o júri

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Agradeço aos meus amigos, aqueles que foram e serão casa longe dela.

palavras-chave *Pinus pinaster*, Regeneração natural, Fogo, Competição, Facilitação.

resumo

As florestas de *Pinus pinaster* estão entre as florestas mais afetadas pelo fogo no Mediterrâneo. O principal objetivo desta dissertação foi estudar a dinâmica natural da regeneração natural do *P. pinaster* em cenário de pós-fogo em três populações do centro costeiro de Portugal, afetadas pelos fogos de 2017. Para isto foram amostrados 18 pontos de amostragem, com duas réplicas cada de áreas de $50m^2$, nas quais foram caracterizados os povoamentos florestais e as estruturas da vegetação de sub-coberto. A densidade da regeneração de pinheiro-bravo foi avaliada em quatro sub-parcelas de $1m^2$ em cada tratamento e população. Foram realizadas ANOVAS para verificar as diferenças entre populações e tratamentos. As correlações entre a regeneração de pinheiro-bravo e as variáveis: bióticas (povoamento, cobertura de solo, coberto vegetal) e abióticas (topografia e clima) foram testadas através do cálculo de correlações de Spearman. Foram registados valores de regeneração considerados sustentáveis nas três populações para os dois tratamentos, onde apenas Mira mostrou indícios de dificuldade de estabelecimento devido às baixas taxas de sobrevivência. Povoamentos mais antigos, com maiores produções de biomassa mostram uma relação negativa com a sobrevivência e altura da regeneração. Foram registados altos níveis de invasão que demonstraram uma relação negativa com a densidade e sobrevivência da regeneração. A altura do coberto invasor demonstrou ser negativa para a sobrevivência, demonstrando assim nos dois casos relações de competição. Verificou-se o efeito contrário relativamente á cobertura de flora nativa onde se registaram relações positivas com a sobrevivência e altura da regeneração demonstrando assim relações de facilitação com a regeneração.

keywords

Pinus pinaster, Natural Regeneration, Fire, Competition, Facilitation.

abstract

Pinus pinaster forests are among the most affected forests by fire in the Mediterranean. The main objective of this dissertation was to study the natural dynamics of *P. pinaster* natural regeneration in a post-fire scenario in three populations of central coastal Portugal, that affected by the 2017 fires (our treatment). For this, 18 sampling points with two replicas, areas of 50m^2 were sampled, in which forest stands and understory vegetation structures were characterized. The density of *P. pinaster* regeneration was assessed in four sub-plots of 1m^2 in each treatment and population. ANOVAs were performed to verify differences between populations and treatments. Correlations between maritime pine regeneration and the study variables: biotic (stand, soil cover and plant cover) and abiotic (topography and climate), were tested by calculating Spearman correlations. Regeneration values considered sustainable were recorded in all three populations for the two treatments, where only Mira showed signs of establishment difficulty due to low survival rates. Older stands, with higher biomass productions, show a negative relationship with survival and height of regeneration. High levels of invasion were recorded which showed a negative relationship with regeneration density and survival. The height of the invasive cover demonstrated to be negative for survival, thus demonstrating in both cases relations of competition. The opposite effect was verified for the cover of native flora where positive relations were registered with survival and height of regeneration, thus demonstrating relations of facilitation with regeneration.

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Supplementary material 4. Graphical representation of all Spearman's correlations tested for the a) unburnt, b) burnt treatments. Negative correlations are shown in red, positive correlations in blue. The X represent p-values>0.05.

1. Introduction

Fire is a natural shaper of Mediterranean ecosystems dynamics having great influence on the biological productivity and on the composition of the ecosystem itself. This creates open areas, which favors the germination and development of several species, changing the content of available nutrients (Calvo *et al.*, 2008). Although, due to the characteristics of the climate that promote the accumulation of biomass, hot and dry summers increases the occurrence of fires, and recurrent forest fires can greatly increase the loss of forest area, can impact ecological communities through direct mortality of individuals (plants and animals) and affect soil quality (Fernández-García *et al.,* 2021). In addition, have occurred recent changes in fire regimes aggravated by the intensification of human activity and climate change (Moreira *et al.,* 2020; Fernández-García *et al.,* 2021). Soils are an essential resource that can suffer significant changes when exposed to fire in terms of physics and biochemical properties (Pereira *et al.,* 2018). Burnt soils are more prone to erosion, being more hydrophobic and less structured (Fernández-García *et al.,* 2021).

Apart from the threats linked to fire, there are other imminent threats to the pine forests. The growing of forest pest and diseases, occurs due to the increase of incidence of forest fires, climate change, as well as the abandonment of rural land and the decreased active forest management (Sousa, 2006). The biotic agents that affect most species are the scolithids (*Tomiscus piniperda, T. destruens, Ips sexdentatus*, and *Orthotomicus erosus*) and the pine processionary, *Thaumetopoea pityocampa* (Sousa, 2016; Sousa *et al.*, 2019). It is also present in Portugal, one of the most dangerous pathogens for conifers - *Bursaphelenchus xylophilus* - responsible for the known pine wilt disease (Sousa *et al.,* 2019).

Also, the ongoing climate change increases the vulnerability of the European forest (Forzieri *et al.,* 2021). Extreme drought events and increased aridity are leading to forest decline and tree mortality (Rodriguez-Vallejo and Navarro-Cerrillo, 2019). Because they are interconnected with various threats, climate change is one of the biggest challenges for forestry in the coming decades as it is expected to drastically change the growing conditions for trees (Forzieri *et al.,* 2021).

1.1 Portuguese Forests

From 1995 to 2010 Portuguese forest decreasing from 37.1% to 35%, a trend that was reversed in 2015 with a growth of 1.9% compared to the year 2010 (ICNF, 2019). When compared to the rest of Europe, Portuguese numbers are discouraging. The European average of public forests is around 76%, but in Portugal, it is only 3% (Forest Europe, 2015), which together with the fact that the properties are very fragmented and the lack of registration of the properties makes it difficult to implement management plans (Santos *et al.,* 2021). Consequently, only 22% of the Portuguese forestry area has management plans (Forest Europe, 2015).

Currently, the forest spaces occupy about 69.4% of the national territory (6.1 million ha) and include the forest, scrub, and unproductive land. At a structural and functional level, the Portuguese forest can be organized into four major groups: resinous (*Pinus pinaster*, *Pinus pinea*), evergreen hardwoods (*montados*, holm oak, cork oak), deciduous hardwoods (oaks, chestnut trees) and, finally, industrial silvous hardwoods (Eucalyptus) (ICNF, 2019). In terms of representativeness, the ones that have the highest occupation are the evergreen hardwoods with 33.7%, followed by pine forests (29.7%), eucalyptus trees (26%), and lastly, deciduous hardwoods with 9.9% (Figure 1).

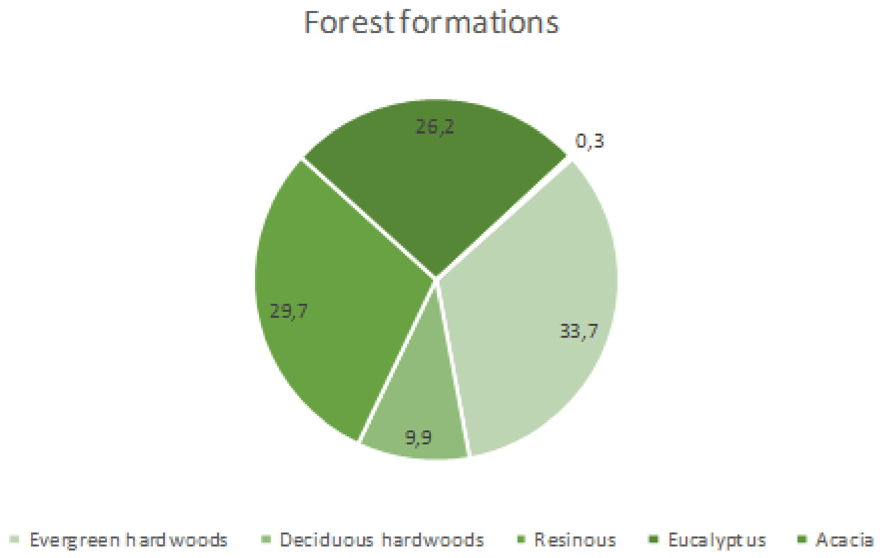


Figure 1. Percentage of forest space occupied by various forest groups [ICNF 2019].

The Portuguese Forest needs new forestry practices to create a more resilient and exploitable biodiversity forest. Therefore, changes in the forest structure should be introduced to reduce its combustibility, in order to better control the fire behavior, and thus decreasing the likelihood of large fires occurring as well as their severity (Fonseca *et al.,* 2019).

Among other measures of preventive forestry, there is the pruning, thinning and the elimination of transitional fuel, creating vertical discontinuities, which decreases the continuity of the canopy fire. To increase the resilience of forest ecosystems it is important to increase their diversity. For this purpose, several measures can be put in place, such as creating a mosaic landscape at the field level where there is a mixture of resinous species with leafy species. This mixing can occur by creating different vertical strata, through strips, or by creating borders with leafy species. Good species for this type of action are considered: strawberry tree (*Arbutus unedo*), faya (*Myrica faya*), cork oak (*Quercus suber*), oak (*Quercus robur*) and laurel (*Laurus nobilis*) (Fonseca *et al*., 2019). The clear-cutting is one of the most used methods of harvesting, however, in the present, the need for new, more sustainable practices that foster natural regeneration, without sacrificing the soil and allowing for large post-logging recruitment, is becoming imperative (Stuiver *et al.*, 2016). Clear-cutting causes disruption to the forest soil and leads to changes in the microclimate, increasing temperature and wind exposure with the removal of trees. This method also generates an impact on water availability for the seedling through differences in evapotranspiration, air humidity, soil moisture and soil properties (Økland *et al.,* 2003; Jerabkova *et al.,* 2011).

1.2 *Pinus pinaster* forest

Pinus pinaster forests occupy a significant part of the western Mediterranean basin (1.5 million ha), covering the Atlantic meso-Mediterranean part of Portugal, also being distributed in France, Italy, Morocco, and Tunisia (De la Heras *et al*., 2002; Vinas *et al.,* 2016). There are 18 subspecies that can be grouped into three groups: Atlantic, Circum-Mediterranean, and Maghreb (Vinas *et al.,* 2016).

Due to its great genetic variability, *Pinus pinaster* is very ecologically versatile, having a wide range of characteristics of growth, resistance, and adaptation to ice and drought (Fernandes and Rigolot, 2007). It can thus be seen that this conifer has a dispersed distribution and can survive under wide ranges of both climate and soil conditions (De la Heras *et al*., 2002). This species prefers siliceous, coarse-textured and acid pH soils, but resists a variable set of soils with basic pH and fine texture and may thrive in poor soils (Jardim Botânico da UTAD, 2019). *Pinus pinaster* grows naturally in warm temperate regions with oceanic influence, preferably in areas where precipitation exceeds 600mm, in an altitude range from the average sea level to 1500m, with the best settlements being found at 400m altitude. This is a heliophile species, thus being intolerant to shade, admitting it only in the first months after germination (Vinas *et al.,* 2016; Jardim botânico da UTAD, 2019). *Pinus pinaster* can reach 40m in height and longevity of 300 years. It is a monoicous tree; its male flowers are yellowish ears that bloom in March and the female flowers are pinecones (Vinas *et al.,* 2016).

Pinus pinaster is an obligate seeder that often responds to fire through a quick dispersion of seeds, that can start during the fire and continuing for a few months after the disturbance (Taboada *et al.,* 2017; Cruz-Alonso *et al.,* 2019) due the germination of canopy seed bank inside serotinous cones (Maia *et al.,* 2012). Serotiny is common among pine species in fire prone ecosystems (Agee, 1998), and is defined as the capacity to retain seeds in long-closed cones within the tree canopy (Gauthier *et al.,* 1996), requiring an environmental stimulus like a heat shock for dispersal seeds (Hernández-Serrano *et al.,* 2013). For *Pinus pinaster*, the average percentage of serotony is around 2-82% in the Iberian populations (Tapias *et al.,* 2004), being highly variable between populations (Pausas *et al.,* 2008). Populations that have a high percentage of serotiny can retain viable seeds in the serotinous cones for decades, thus having a greater probability of surviving more severe canopy fires, ensuring successful post-fire regeneration (Tapias *et al.*, 2004; Corticeiro, n.d). The seed germination and seedling recruitment are well-adapted to exploit post-fire conditions (Rego, 1991; Luis-Calabuig *et al.,* 2002) but the Portuguese Coastal maritime pine populations have in general a low serotinous (Tapias e*t al.*, 2001; Corticeiro *et al.*, n.d).

In Portugal, being the second-largest forest formation, the pine forest is a great asset in terms of forest resources. *Pinus pinaster* is the most representative resinous, thus having a high contribution to the Portuguese economy. In 2018, in Portugal, the pine sector represented 88% of companies and 80% of jobs with an export volume of 35% (INE, 2020). Among the production objectives of this resinous are pellets, paper pulp, the creation of poles, use of bark as substrate and resin production (Jardim botânico da UTAD, 2019; Sousa *et al.,* 2019). The resin was one of the most important by-products in 2018 (Centro Pinus, 2019). This by-product may be used in various industries such as waxes, thinner, varnishes and even in the pharmaceutical industry. Syrups or infusions can be prepared from their buds to treat bronchitis and other respiratory diseases due to their antiseptic and expectorant action (Grunwald and Janicke, 2009).

Also, ecologically, *Pinus pinaster* has very important functions. It has been used for dune stabilization, agricultural wind, and salt spray protection in coastal crops (Vinas *et al.,* 2016). Due to its tolerance to poor soils, it has been used in soil conservation and erosion protection, because of its ease in creating mycorrhizal networks that increase soil quality and stability. It should also be noted that the pine forest ecosystem is favorable to the development of fungi such as *Boletus spp* and *Lactarius spp* mushrooms, creating space for more profit coming from the pinewood production (De la Heras *et al.,* 2002; Vinas *et al.,* 2016).

1.3 Threats to Portuguese forest

1.3.1 Climate change

Land use and climate interact in complex ways through physical, biological, and geochemical forces and feedbacks at different time and space scales. The increase in climatic extremes, such as heatwaves and periods of heat, can lead to the detriment of forest ecosystems, leading to a decrease not only in productivity but also in biodiversity, since long periods of drought can cause the loss of diversity of shrub species in Mediterranean ecosystems (Sánchez-Salguero *et al.*, 2018). Although this type of ecosystem is already adapted to seasonal periods of drought and irregular rainfall regimes, extreme weather events can lead to changes in the tolerance of some species (Gazol *et al.*, 2018; Alegria *et al.,* 2020).

Climate change will have a strong impact on plants, since precipitation and temperature not only influence their growth and survival, but also shape their distribution (Santos *et al.,* 2006; Alegria *et al.*, 2020). A shift towards warmer and drier climate could threaten many pine plantations in the Mediterranean region (Vicente-serrano *et al.,* 2015). (Rodriguez-Vallejo *et al.,* 2021) shows a link between the high vulnerability to drought in *P. pinaster* planted forests and the plantations dieback, increased the tree mortality. These changes are of particular concern in younger plants, which are more sensitive to climatic variations, since they do not yet have their root system fully developed, decreasing the absorption capacity. Adult trees are more tolerant, due to the existence of a reserve, but also because their root system is fully developed and allows to increase the absorption capacity if necessary (Alegria *et al*., 2020). In winter, low temperatures limit plant productivity, photosynthesis is restricted, and xylem embolism can occur (Gazol *et al.,* 2020). In coastal pine forest areas, the climate is not extreme, which is why some stress factors in this ecosystem are not so evident.

For the reasons described above, pine regeneration can be especially sensitive to climate change and can be limited by periods of drought. Summer rains are, therefore, indicated as advantageous for the germination and initial development of the maritime pine. In this way, it is indicated that management plans consider the climatic conditions of the site, which can strongly affect the regeneration density, as well as its development and establishment (Rodríguez-García *et al.,* 2011).

1.3.2. Invasive species

In Portugal, there are more than 670 exotic species and about 8% of them have invasive behavior (Marchante *et al.,* 2014). The most abundant species belong to the genus *Acacia*, the most common being *Acacia longifolia* and *Acacia dealbata*, which are already distributed in all forest formations, with invasion values for the maritime pine forests of 14% (IFN6, 2015).

Invasive species alter the structure and the functioning of ecosystems and inherent ecological networks (Marchante *et al.,* 2014), thus being one of the main threats to forest resources and biodiversity in Portugal (Marchante *et al.,* 2018). These cause changes at the level: of the nutrient cycle, as they alter the input of organic matter, causing changes in the breakdown and release of nutrients; the hydrological cycle as its roots can extract considerable volumes of water, changing soil moisture and changing evapotranspiration rates (Jesus *et al.,* 2013); Changes in fauna and flora, as they can lead to fragmentation and loss of habitats (Marchante, 2001); Changes in the fire regime, as they are capable of altering fuel properties, creating favorable characteristics for the occurrence of fires (Jesus *et al.,* 2013). The high growth rate of acacias leads to the accumulation of high fuel loads, which can aggravate the intensity and extent of forest fires. After a fire, the first plants that appear are exactly the invasive species, because of the germination stimulus of seeds deposited in the first layer of soil, contributing, and reinforcing again to the increased susceptibility of the area to new fires.

1.4 Threats relate to fire

Since the 1970s, fires have gained importance in Mediterranean biomes. This is due to changes in the composition and management of forests, but also to the abandonment of agricultural land (Pausas *et al*., 2008; Fernandes *et al.,* 2010). The year of 2017 was a historical landmark for Portugal, with 442,418 ha of burnt area, of which 164,167 ha are pine forests, and thus having 428% more burnt area than the annual average. After a long period of drought, from the exposure to hot air masses coming from North Africa, on October 15 appeared the tropical storm Ophelia, which raised the meteorological danger of fire (ICNF, 2019). Among the most affected areas we find the Mata Nacional de Pedrogão with 89.6% of your area burnt, and the Mata Nacional de Leiria with 86.0% (ICNF, 2019).

Fire severity is important for ecosystem processes and forest management. It describes the immediate effects of fire, related to the degree of removal and/or destruction of biomass by the fire. Also, fire severity played a significant role in the pine recruitment, lower pine recruitment following a higher fire severity (Maia *et al.,* 2012). The evaluation is qualitatively measured in separate classes (litter and vegetation) based on visual effects such as the amount of ash present and the condition of the branches (Keeley, 2009; Fernandes *et al.,* 2010) According to the classification of (Botella-Martínez and Fernandéz-Manso, 2017) this is divided into three classes, low (mostly crowns unaffected), moderate (predominantly scorched crowns) and high (predominantly burnt crowns). Fire severity depends on the interaction of the fire behavior with the vegetation structure, especially with the stratification level and vertical discontinuity (Fernandes and Guiomar, 2019). The response to the severity will depend on the type of ecosystem in which the fire occurs. High severity can lead to processes of soil erosion, habitat fragmentation, invasion, and changes in the amount of carbon available. The climate, composition, and structure of the forest, plus the topography are the factors that most influence the fire regimes. The climate is the main force in terms of fire space and duration, while vegetation and topography control fire behavior (Fernandes *et al.,* 2010).

1.5 Post-fire forest recovery

The main strategies used by maritime pine are resistance and evasion. Resistance is mainly linked to the distance between the base and the crown and to the thickness of the cork cambium (Agee, 1998; Fernandes *et al.*, 2005). In case of fire, it is favorable that a high distance between the base and the canopy exists for the preservation of the aerial seed bank, which explains why in dense populations *Pinus pinaster* promotes the death of its lower branches, making it difficult to spread canopy fire (Agee 1993). It also carries the characteristic of resistance of bark's high thickness that isolates the cambium and protects it from temperature spikes (Hensgt and Dawson, 1994). Concerning the evasion capacity, it is linked to the degree of the percentage of serotiny, this percentage is closely linked with the intensity and severity of the fires, as well as with their recurrence, and varies according to the population (Gauthier, 1996). By preserving the seeds in the aerial bank through this characteristic, not only is a post-fire recruitment impulse obtained, but a greater number of seeds is guaranteed, and with a greater weight and a higher germination rate, since they have protection against competition and predation (Saracino *et al.*, 2004).

It is known that the interaction between provenance, fire severity, post-fire management can be critical for seedling and recruitment success (Vega *et al.*, 2010). Greater severity of fire leads to greater consumption of canopy, decreasing the number of seeds, viability, and emergence (De la Heras *et al.,* 2002). Also, when the severity of the fire is high, there is a great loss of organic matter, making the soil poorer, with a lower acid pH due to the denaturation of organic acids. It also causes changes in the microfauna and microbiome, which hinders the recovery of vegetation. In addition, the ashes that come from this type of severity modify the soil profile, changing the porosity and the water infiltration capacity, favoring runoff phenomena

(Pereira *et al.*, 2018). In fires with moderate severity as the most of Portuguese fires, the pH becomes more basic because the capacity of ash to neutralize soil due their content of K, Ca, and Mg (Certini, 2005).

The litter layer is also related to the severity, fires of greater severity generate more destruction of dead matter, which can be a key for the success of the regeneration since the litter presents a positive correlation with post-fire regeneration (Rodriguez-Garcia *et al*., 2010). Studies indicate that this can enhance recruitment since it helps in the accumulation of seeds, provides organic matter, protects against granivores, reducing water-loss and absorbing thermal oscillations (Fernández *et al.,* 2008; Rodriguez-Garcia *et al.,* 2010), but other authors such as (Loydi *et al*., 2013) argue that the litter can act as a barrier for the seed to reach the soil and can also contribute to the infection of seeds by pathogens. The same author suggests that the role of the litter will act according to the climate of the place of interest, in arid climates, the litter may enhance regeneration since it creates humidity (a limiting condition in these ecosystems), whereas in humid climates this may be a conditioning factor for the reasons described above.

Very important as well for regeneration is the dynamics of the companion vegetation. The understory vegetation in western Iberian has a key role in the ecosystem, due the role of water regulation and erosion protection (Maia *et al.,* 2012). The variety of the flora composition can be explained by edaphic and topographic conditions, that limit their native biogeographical range. This type of forest have a great diversity of flora, mainly composed of Ericaceae (*Erica* and *Calluna* genera), Cistaceae (*Cistus monspeliens, Halimium*) and Fabaceae (*Genista tridentata, Stauracanthus sp., Ulex spp.*) (Maia, 2014). There is a bushy composition of ecological interest as *Corema album, Juniperus turbinata, Arbustus unedo*, depending on biogeographical vegetation unit and the edaphic climatic conditions (Centro Pinus, 2020). Regeneration patterns can be different according to the different types of understory that exist at the site, depending on the species present, natural succession is affected through interspecific competition (De la Heras *et al.,* 2002).

Post-fire management is also described as one of the key factors for successful regeneration. In places where there is reproductive potential, but there is no recruitment establishment, the biggest cause of mortality is salvage logging of burnt wood (Fernández *et al.*, 2008; Maia *et al*., 2012). In the case of not having occurred crown fire, cutting and thinning can favor the emergence of new seedlings, since through the handling of trees new seeds can be released, at the same time that clearings will be created, providing better conditions for the emergency (Vega *et al*., 2010).

It is very important that in the burnt areas the management is done with the intention of increasing the natural regeneration, due the genetic conservation and the edaphic climatic adaptations, that will allow for more vigorous growth (Rodríguez García *et al.*, 2007). Depending on the success rate of regeneration there are usually three action lines to consider: do not move, promote regeneration and active reforestation. Forest recovery after the fire is usually rapid, with a high recruitment density that is sometimes excessive for the viability of tree establishment due to intraspecific competition. When this is the case, it is important to practice forestry measures such as thinning and pruning, to decrease intraspecific competition and favoring regeneration. If the fire recurrence is longer than the time for the trees to reach maturity, sowing may be performed. In this process, seeds from the same area of origin should be chosen, preferably from untouched contiguous areas, so as not to introduce genetic contamination and to ensure phenotypic quality and yield potential. If sowing does not work, reforestation may also be carried out with genetically improved plants or enriched with mycorrhizal formations for an easier establishment (De la Heras *et al.,* 2002).

2. Objetives

Pinus pinaster forests are amongst the most affected by wildfires in the Mediterranean ecosystems (Madrigal et al. 2010). In Portugal, maritime pine is the second-largest forest type, and is the forest species that have the highest accumulation of total biomass and therefore the largest amount of carbon stored in mainland Portugal, with a great value for economic, recreational, soil and biodiversity conservation purposes (De la Heras *et al.,* 2012; ICNF, 2015).

The main objective to this study is to further the knowledge of the dynamics of natural regeneration of *Pinus pinaster* populations, from recruitment to establishment, in the presence and absence of stand replacing fires. For this purpose, the natural regeneration of maritime pine was assessed along a latitudinal gradient in a forest inventory was made in three populations of the Portuguese coastal center (Mira, Tocha, Leiria). The specific objectives were then to, to 1) investigate natural regeneration of maritime pine in three different populations: Mira, Tocha and Leiria and 2) evaluate how fire and specific site conditions affect natural regeneration potential and the establishment of regeneration.

The final aim of this work is to contribute to the predictability of the potential of natural regeneration of pine forests into creating natural exploitable forests, under different disturbance scenarios (fire occurrence) and climatic conditions.

3. Materials and methods

3.1 Study area Location

Three *Pinus pinaster* populations, located along the central coast of Portugal (Figure 2) were selected to investigate the natural regeneration of *Pinus pinaster*: Mira, Tocha, and Leiria, along a geographic gradient of about 140 km from North to South of Portugal (Mira to Leiria). These populations were strongly affected by the fires of October 2017, with 55-86% of their area being burned with fire severities varying from low and extreme (ICNF 2019). Each selected population included unburnt and burnt areas. This area belongs to the Western Mesozoic Front, with soils that vary between regosols and podzols, with coarse sandy textures, characterized for low organic matter contents and low water retention (ICFN 2010; ICNF 2014).

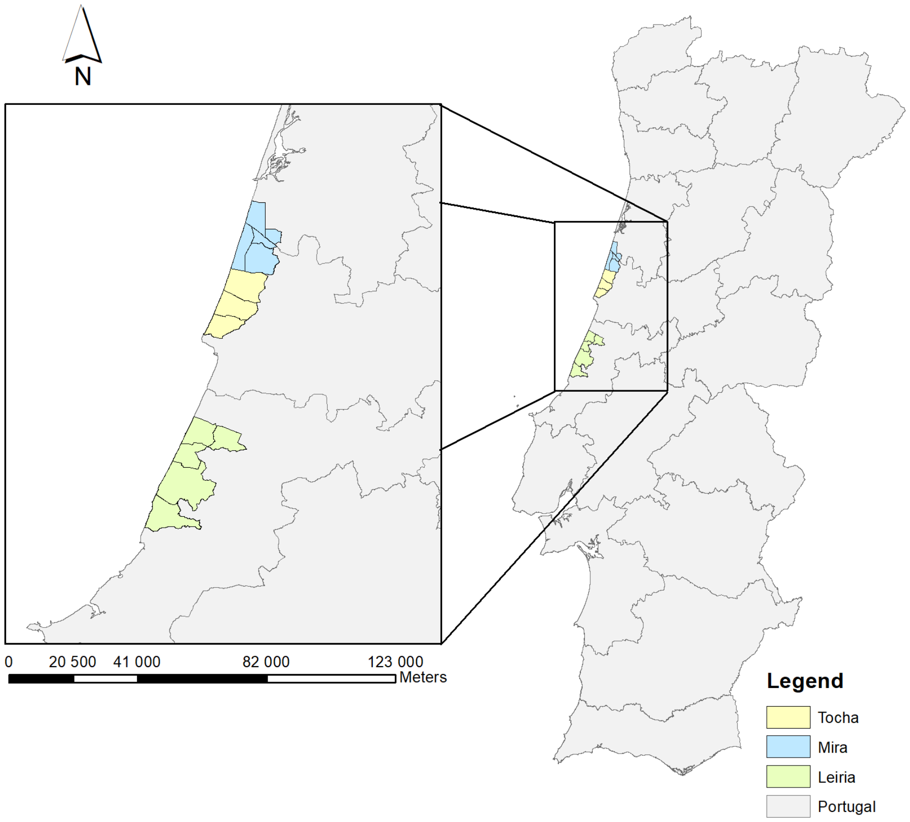


Figure 2 - Selected Pinus pinaster populations Mira (M), Tocha (T) and Leiria (L), distributed from North to South.

3.2 Field Sampling

A total of 18 sampling points were sampled between the summer of 2019 and the spring of 2020. For each sampling point, two circular plots were marked, making a total of 36 replicas. The first replica was marked 20 meters after the first pine tree counted after the road line; the second one was marked around 40 meters away from the center of the first plot, depending on the conditions of the terrain. Machinery paths and marks were avoided, to avoid introducing variability. All points were chosen in this way to avoid bias in the choice of the sample.

In each circular plot of 50 m^2 (Maia *et al.,* 2014) the forest inventory was carried out, with the coordinates marked in the center circumference (Figure 3). In each axis, micro-plots with an area of 1 m^2 were marked, always 2m away from the center of the circle, where the regeneration of the pine was measured. For the measurement of the micro-plots, an order was established, where the first to be marked was with our backs facing the road and then following a clockwise direction.

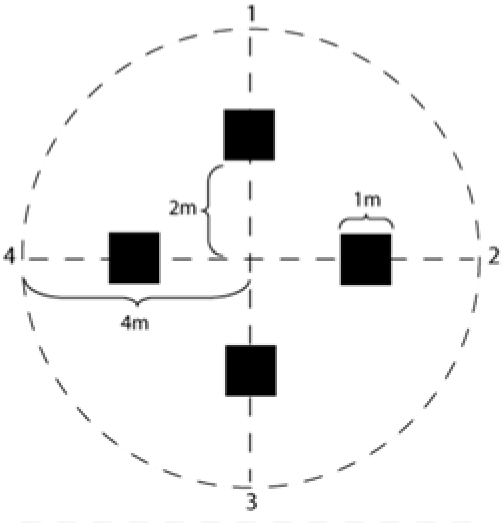


Figure 3. Schematic view of a sampling plot and its four sub-plots

3.3. Study variables

3.3.1 Abiotic variables – Topography and Climate

In topographical terms, these areas are mostly flat with a variation between -10m and 73m above sea level slope inclination between 0% and 32% (ICNF 2014). The height above sea level was automatically calculated with the GPS by the coordinates measured in the center of the sampling point. Distance to the sea was obtained from measurements in straight line in satellite imagery (Google Earth), between the central location of the plot and the sea line using the ruler tool.

The study populations belong to the Mediterranean climate zone Csb, with a temperate climate, dry and hot summers (IPMA, 2020). Annual climate data (average, maximum, and minimum temperature; average precipitation, days without rain, days with a temperature above 35°C), were obtained from nearby weather stations. Climatic data of the first two years after fire was obtained from nearby weather stations (IPMA, 2020). The first year began to be counted from the month following the date of the fire, being thus from November 2017 to October 2018. The second year was defined as the period from November 2018 to October 2019. The two years were considered due to the year of formation and maturation of *P. pinaster* cones and respective seeds (UNAC, 2014). To estimate periods of water stress, days with temperatures above 35°C were selected and the periods of days without rain.

3.3.2 Biotic variables

3.3.2.1 Stand Characterization

The forest inventory was prepared in such a way as to be carried out in a systematic way, to be more accurate and with random sampling to avoid systematic errors. It was carried out in circular plots of fixed area (50 m^2). In this inventory (Supplementary material 1), the number of trees per quadrant was counted to calculate the density of trees; then, the perimeter was measured using a tape measure, at 1.30 m from the ground (breast heigth), for later conversion to DBH (diameter at breast heigth) and basal area through dendometric equations. As there are no available records of planting/sowing, stand age was estimated by the method of counting whorls of branches of the trees to estimate the average age of the stands. The topography of the site was characterized, and evidence of forest management was noted and registered out based on direct observations of field evidence (vegetation control, thinning, soil mobilization).

3.3.2.2 Soil Cover and Vegetation structure

The percentage of other soil cover features was estimated, in the following classes - bare soil, litter, herbaceous that covering the soil, mosses, and lichens - and in burnt areas, the percentage of ash was also estimated.

The evaluation of the plant community was based on quantitative and qualitative criteria, the first relates to abundance and coverage and the second to composition (Carvalho 1994). The vegetation cover was also measured in percentage and the vegetation cover was estimated in its total, native, and invasive and divided by strata. The height of each category was estimated for the entire plot. 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 species (DL 92/2019). Hence, the following categories were considered

A) Total native plant cover – subdivided into trees, shrubs, herbaceous

B) Total invasive cover - invasive trees, shrubs, invasive herbaceous.

3.3.2.3 Regeneration of *Pinus pinaster*

In a second approach, for the study of regeneration were sampled a total of 144 micro-plots, where the number of live and dead seedlings of *Pinus pinaster* was assessed to calculate the survival rate and the density per m^2. In addition to the number, the height from the root neck to the last whorl and the age of the seedlings was also estimated through whorl counting, to characterize the regeneration cohorts. In this approach, the number of pinecones per m^2 was also counted as a proxy of seed availability.

3.4 Statistical analysis

To test the differences between treatment and populations, normality tests (Shapiro test) and homogeneity of variances (Levene test or bartlett test) were performed for later analysis of ANOVA (in case of normality and homogeneity), or the Kruskal-Wallis test when one of these principles was broken. Except for the differences between tree age, DHB, age regeneration and density of pinecones, variables in which Tuckey tests were applied, for all others pairwise Wilcoxon tests were used.

Since the data under analysis did not show normality, Spearman's correlations were calculated, where Rho values above 0,35 are accepted due to the great variability of the field data. For significance value, a p-value≤0.05 was accepted. All correlations between density of regeneration, survival, and height of regeneration were tested against all variables under study – the abiotic (Elevation (m); Distance to sea (m); Mean annual precipitation (mm); Cumulative precipitation of March (mm); Mean annual temperature (C°); Maximum temperature (C°); Minimum Temperature (C°); Consecutive days without rain; Number of days with temperature superior 35C°) and the biotic (% Bare soil; % Litter; % Mosses and Lichens; % Herbaceous that cover the soil; % Soil cover; % invasive trees; %invasive herbaceous; %invasive cover % Shrub cover; % Herbaceous cover; % Tree cover; % Native cover; % Total cover; Average cover height – total, native, and invasive) for both control and burn treatment. Data analysis was performed on R 4.1.1 software.

4. Results

4.1 Topography and Climate

The three areas are considered generally flat, their average height above the sea varying between 21.3m in Mira burnt and 64.9m in Leiria unburnt (Table 1). Distance from the sea these values vary between 670m and 4880m, on average Leiria burnt is the population with the shortest distance to the sea (1,9km) and Mira burnt the most distant population (4,4km).

Table 1. Topographic characterization of the selected populations, for the unburnt (U) and burnt (B) areas.

	Topographic features (mean ± sd)			
	Above sea level (m)		**Distance to sea (m)**	
	U	**B**	**U**	**B**
Mira	29.2±5	21.3±15.7	2011±425	4408±1179
Tocha	57.2±21.2	50±10	3191±349	3715±565
Leiria	64.9±6.4	49±15	2027±1549	1936±509

Although there are no significant differences in average annual precipitation, this value in the first year was lower when compared to the second year. During the first year, mean annual precipitation varied from 952 mm at Leiria to 1115 mm at Mira (Supplementary material 2). In the second year, the lowest value of precipitation was registered at Leiria, 742 mm/year, while the highest was verified in Mira, 960 mm/year. There was a decreasing north-south gradient in the average annual rainfall for both years, with Mira being the place where it rains most, and Leiria the place where it rains least.

Precipitation distribution was mainly concentrated in the moths of March, April, and November for both years in the three locations. Due to the influence of precipitation in the months of March, April and May (time of cone and seed formation) on the productivity of the pinecones (Gordo, 2004; Mutke *et al.*, 2005), were analysed the precipitations of those months were analysed separately. March is the month with major precipitation accumulation and follows the trend of average annual precipitation, where there is a decreasing north-south precipitation gradient. This was the only one of the spring months where there were significant differences between the years (Mira F=14.11; Tocha F=14.87, and Leiria F=15.58; p-value<0.05). In order to find periods of dryness, the maximum of consecutive days without rain were counted, being in the first year 98 days in Mira and Tocha and 100 days in Leiria; in the second year, this period was 30 days in Mira and Tocha and 35 days in Leiria, being this period much shorter in the second year. In the first year, this period was recorded between the beginning of July and the beginning of October, coinciding with the period of higher temperatures (23-41°C). In the second year, this period was located between August and September with average temperatures in the range of 25-34°C.

There are no significant differences related to mean temperature between years neither between populations. The mean temperatures varying between 15.2°C (Tocha) and 15.4°C (Leiria) in the first year, and from 15.5°C (Tocha) and 15.7°C (Leiria) in the second year (Table 2). The maximum temperatures recorded in the first year were higher than in the second year to the three locations. In the first year the maximum temperature was recorded in August for the three locations (39.9°C in Mira; 40.5°C in Tocha and 40.8°C in Leiria), in the second year in September the three locations were also recorded (34.9°C in Mira; 35.4°C in Tocha and 35.8°C in Leiria). Considering the number of consecutive days with temperature records equal or above 35°C, the period was longer for the three locations during the first year, which was verified for the three localities in the beginning and middle and August for periods of 4/3 days, respectively. In the second year, there were no consecutive days with temperatures over 35°C in the three locations. For the minimum temperatures recorded, only Mira registered temperatures below 0°C, only one day a year, being of -1°C in February in the first year.

Table 2. Climate characterization for the three populations, segmented by the first (November 2017- October 2018) and second (November 2018- October 2019), year after fire for the populations in study. The different letter indicates significant differences (p<0.05)

	Climatic characterization													
	Mean Annual precipitation (mm)		**Precipitation accumulation in March (mm)**		**Mean Temperature C°**		**Maximum Temperature C°**		**Minimum Temperature C°**		**Max consecutive days without rain**		**Nº of Days with T⩾35°**	
Year	**1º**	**2º**	**1º**	**2º**	**1º**	**2º**	**1º**	**2º**	**1º**	**2º**	**1º**	**2º**	**1º**	**2º**
Mira	1115$^{(a)}$	960$^{(a)}$	275$^{(a)}$	65$^{(b)}$	15.3$^{(a)}$	15.6$^{(a)}$	39.9	34.9	-1	-0.5	98	30	9	4
Tocha	1082$^{(a)}$	832$^{(a)}$	268$^{(a)}$	61$^{(b)}$	15.2$^{(a)}$	15.5$^{(a)}$	40.5	35.4	0.2	1	98	30	9	3
Leiria	952$^{(a)}$	741$^{(a)}$	254$^{(a)}$	57$^{(b)}$	15.4$^{(a)}$	15.7$^{(a)}$	40.8	35.8	0.9	1.6	100	35	10	1

4.1 Stand characterization

Data related to stand's characterization was collected in *P. pinaster* stands of Leiria, Tocha and Mira, by installing inventory plots in burnt and unburnt treatment stands, as described in point 3.3.2.1 of the section "Materials and methods".

In the unburnt treatment, the population with the highest average age was from Tocha (47 years old) and the lowest was from Mira with an average age of 41 years. In the burned treatment, Mira was the oldest population, 40 years old, and while Leiria was the youngest (32 years) (Table 3). There were significant differences between the age of different populations in the burnt treatment ($F= 8.619$; $p\text{-value}<0.05$). Analysing the differences between treatments, Leiria ($\chi 2= 17.9$; $p\text{-value}<0.05$) and Tocha ($\chi 2= 15.13$; $p\text{-value}<0.05$) showed significant differences concerning the age of the unburnt and of burnt stands within the same population.

When considering trees for density, the existing trunks were counted regardless of the viability of the trees. Regarding stand's density in the unburnt treatment, Tocha had the highest average density (1007.2 tree/ha) while the lowest was found in Leiria with 770.2 tree/ha. The burnt treatment followed the same pattern with the highest stand density found in Tocha (2192 tree/ha) and the lowest in Leiria with 1214.4 tree/ha. There were no significant differences between populations for the density of *P. pinaster* stands. In terms of treatment, Mira ($\chi 2= 5.95$; $p\text{-value}<0.05$) and Tocha ($\chi 2= 6.18$; $p\text{-value}<0.05$) presented significant differences between treatments, with higher densities being observed in burned stands within the same population.

By analysing biomass production on the three populations, significant differences were found between populations in the unburnt treatment ($F= 7.20$; $p\text{-value}<0.05$) and in the burnt treatment ($\chi 2= 5.22$; $p\text{-value}<0.05$). Tocha had the thicker trees in the burnt treatment, with an average of DHB of 28.7 cm, whereas Leiria had the thinner trees (13.9 cm). In the unburnt treatment the ticker trees are in Tocha (28.7 cm) and the thinner trees are in Mira (23.9 cm). There were significant DHB differences between treatments for Mira ($\chi 2= 13.91$; $p\text{-value}<0.05$), Tocha ($\chi 2= 53.3$; $p\text{-value}<0.05$) and Leiria ($\chi 2= 36.51$; $p\text{-value}<0.05$), being the highest values found in Mira and the lowest found in Leiria.

The basal area integrates the density of the population with the basal diameter, thus allowing an estimate to be made of the biomass produced per hectare of forest (Alegria, 2004). In unburnt treatment, there were significant differences between populations ($\chi 2= 5.74$; $p\text{-value}<0.05$). The site with the highest biomass production was the Tocha (0,3986 m^2/ha) and the lowest was Mira (0.2921 m^2/ha). On the other hand, Mira (0.2800 m^2/ha) had highest levels of biomass in the burned treatment, while Leiria had the lowest biomass production of all populations (0.1337 m^2/ha). If we analyse by population, the highest average of basal area was in Tocha (0.2914 m^2/ha) and the lowest was Leiria (0.2224 m^2/ha). Among treatments, there were significant differences in the Tocha (χ2= 6.545; p-value<0.05), with basal areas of 0.3986 m^2/ha in unburnt treatment and 0.1843 m^2/ha in burnt treatment.

Table 3. Stand characterization (mean±sd) on the three locations- Mira, Tocha and Leiria – in unburnt (U) and burnt (B) treatments. The different letter indicates significant differences (p<0.05).

	Stand							
	Age (years)		**Density (tree/ha)**		**DBH (cm)**		**Basal Area (m^2/ha)**	
	U	**B**	**U**	**B**	**U**	**B**	**U**	**B**
Mira	41±8 (abc)	40±8 (bc)	977.6±351.0$^{(a)}$	1807.0±555.5$^{(b)}$	23.9±7.2 (b)	17.3±7.2 (c)	0.2921±0.0643$^{(b)}$	0.2800±0.1299$^{(abc)}$
Tocha	47±6 (a)	37±11 (cd)	1007.2±383.9$^{(a)}$	2192.0±671.4$^{(b)}$	28.7±8.3 (ab)	12.2±5.4 (d)	0.3986±0.084$^{(c)}$	0.1843±0.039$^{(a)}$
Leiria	46±5 (ab)	32±16 (d)	770.2±367.2$^{(a)}$	1214.4±749.2$^{(ab)}$	25.4±6.6 (a)	13.9±7.6 (cd)	0.3143±0.1294$^{(abc)}$	0.1337±0.0724$^{(a)}$

4.2 Soil cover

The most strongly represented category in both treatments and in all populations is littler. The amount of litter was always higher in the unburnt treatment. In Leiria we found the highest percentage of litter (81%) of the unburnt stands, and the lowest was observed in Tocha (64%) (Table 4). The opposite trend is registered with bare soil, always with higher average percentages in the burned treatments than in unburnt treatment, being greater represented in the Tocha with 39% and less in Mira with only 9%. Regarding the average ash coverage, only sampled in burning, it appears in a higher percentage in Tocha with 12% and in a lower percentage in Leiria with only 1%. As for the herbaceous that cover the soil, in unburnt treatment they are more represented in the Tocha (16%) and less in Mira (4%). Mosses and lichens do not appear in Mira in the burnt treatment and the place where they appear with a higher average percentage is on Tocha in the unburnt treatment (18%).

Table 4. Soil cover (%) (mean±sd) on the three locations- Mira, Tocha and Leiria – in unburnt (U) and burnt (B) treatments. The different letter indicates significant differences (p<0.05).

	Soil cover (%)					
	Mira		**Tocha**		**Leiria**	
	U	**U**	**U**	**B**	**U**	**B**
Litter	$79\pm15^{(b)}$	$66\pm2^{(bc)}$	$64\pm22^{(bc)}$	$38\pm27^{(ac)}$	$81\pm10^{(b)}$	$17\pm22^{(a)}$
Bare soil	$1\pm1^{(cd)}$	$9\pm7^{(a)}$	$1\pm1^{(d)}$	$39\pm27^{(b)}$	$0\pm0^{(c)}$	$24\pm23^{(ab)}$
Ash	$0\pm0^{(b)}$	$8\pm10^{(c)}$	$0\pm0^{(b)}$	$12\pm8^{(c)}$	$0\pm0^{(b)}$	$1\pm1^{(a)}$
Herbaceous	$4\pm4^{(d)}$	$25\pm18^{(c)}$	$16\pm18^{(bcd)}$	$8\pm12^{(bd)}$	$13\pm3^{(b)}$	$56\pm25^{(a)}$
Mosses and lichens	$16\pm17^{(b)}$	$0^{(a)}$	$18\pm11^{(b)}$	$3\pm5^{(a)}$	$6\pm9^{(ab)}$	$2\pm4^{(a)}$

In unburnt plots, there were significant differences in bare soil cover (χ2= 7.34; p-value<0.05), and herbaceous that covers the soil (χ2= 6.09; p-value<0.05). In burnt, there are significant differences between populations for litter (χ2= 7.93; p-value<0.05); Bare soil (χ2= 5.85; p-value<0.05); herbaceous that cover the soil (χ2= 11.37; p-value<0.05) and ashes (χ2= 4.09; p-value<0.05)

In Mira, the most represented type of covering is litter in both treatments (79% in unburnt, 62% in burnt), the least represented typology is bare soil equally for both treatments (1% in unburnt, 9% in burnt). Here, there are significant differences in coverage between treatments for bare soil (χ2=8.96; p-value<0.05); herbaceous that cover the soil (χ2=8.36; p-value<0.05) and mosses and lichens (F=5.12; p-value<0.05). In Tocha, the most represented typology remains the litter (64% in unburnt, 38% in burnt) and the least is the herbaceous (16% in unburnt, 8% in burnt). Tocha has the greater cover of mosses and lichens than the other locations. In Tocha, there are differences for bare soil (χ2=8.93; p-value<0.05) and mosses and lichens (χ2=6.40; p-value<0.05). Leiria also has litter as the most representative typology (81% in unburnt, 16% in burnt), and the least it is the mosses and lichens (6% in unburnt, 2% in burnt). Leiria has a greater representativeness of herbaceous than the other locations. In Leiria, there are differences for litter (χ2=8.33; p-value<0.05); bare soil (χ2= 6.70; p-value<0.05), and herbaceous that cover the soil (χ2= 8.36; p-value<0.05).

4.3. Plant cover

4.3.1 Total plant cover

According to in situ observations, the most common families are Leguminosae, Cistaceae, Ericaceae, and Asteraceae. The most common species of each family are - *Acacia longifolia* and *Ulex spp.* (Leguminosae); *Cistus salvifolius* and *Halimium halimifolium subsp. Multiflorum* (Cistaceae); *Arbustus unedo* and *Calluna vulgaris* (Ericaceae), and *Senecio lividus* and *Conyza canadensis* (Asteraceae). Analyzing by strata, the most common tree species are the invasive *A. longifolia* and the native *A. unedo*. The most common shrubs *C. salvifolius, Ulex spp. H. halimifolium* and *C. vulgaris*, while in herbaceous the most common are *S. lividus* and the invasive *C. canadensis.* There are different strategies for post-fire reproduction among the families. The species belonging to Ericaceae included reprousters and seeders, Cistaceae and Asteraceae are seeders and Leguminosae are, in that case, seeders. Oher species of ecological interest were also found, such as *Corema album*, *Myrica faya*, *Ruscus aculeatos*, *Cytisus multiflorus, Halimium calycinum, Lavandula stoechas*, *Daphne gnidum* and other species of *Cistus* genus (*C. lanidifer and C. psilosepalus*), although less frequently. The presence of other invaders has also been identified, such as *Acacia dealbata* and *Carpobrotus edulis.*

The place with the highest total vegetation cover in unburnt treatment is Mira (45%) and the lowest is Tocha (38%). In brunt treatment the highest coverage is in Leiria (87%) and the place with the lowest coverage Tocha (34%) (Table 5; Figure 4). The total coverage was divided into native and invasive.

As for the percentage of total native coverage in unburnt it is the Tocha that presented the highest average (37%), the lowest for the least treatment is in Mira with only 19%, thus being the only place/treatment in which the average percentage of coverage invasive species is superior to native coverage. In the burnt treatment, the place with the largest native coverage is Leiria (80%) and the one with the lowest is the Tocha with 19%. Native vegetation cover was divided into three categories, in which the percentage of coverage (shrubs, herbaceous and other trees) were measured. The category most represented in all places and treatments is the shrubs, with the burned treatment having the highest coverage in Leiria (76%) and the lowest in Tocha (15%) (Table 5). Trees are the least represented group for both treatments. In unburnt, the place with the highest average tree coverage is the Tocha (4%) and the lowest is Mira, with no trees other than pine. In the burnt treatment, the place with the highest coverage is Leiria with 1% and the lowest Mira, which does not register other trees. Except for Tocha (19%), the remaining locations had greater native vegetation coverage at burnt plots (Mira 33% and Leiria 80%). Except for the

Tocha, the burnt treatment has a total coverage greater than unburnt, 50% in Mira and Leiria 87%.

Table 5. Native vegetal cover characterization (mean±sd) by typology on the three locations- Mira, Tocha and Leiria – in unburnt (U) and burnt (B) treatments. The different letter indicates significant differences (p<0.05).

	Vegetal cover (%)					
	Mira		**Tocha**		**Leiria**	
	U	**B**	**U**	**B**	**U**	**B**
Total cover	$45\pm20^{(bc)}$	$50\pm13^{(b)}$	$38\pm16^{(bc)}$	$34\pm11^{(c)}$	$39\pm15^{(bc)}$	$87\pm18^{(a)}$
Native cover	$19\pm13^{(b)}$	$33\pm8^{(c)}$	$37\pm17^{(b)}$	$19\pm16^{(c)}$	$29\pm18^{(b)}$	$80\pm15^{(a)}$
Shrubs	$16\pm15^{(b)}$	$20\pm12^{(b)}$	$31\pm22^{(b)}$	$15\pm12^{(b)}$	$20\pm21^{(b)}$	$76\pm1^{(a)}$
Herbaceous	$3\pm5^{(a)}$	$13\pm12^{(a)}$	$2\pm2^{(a)}$	$4\pm7^{(a)}$	$8\pm8^{(a)}$	$3\pm3^{(a)}$
Trees	$0\pm0^{(b)}$	$0\pm0^{(ab)}$	$4\pm5^{(b)}$	$0\pm0^{(a)}$	$1\pm2^{(b)}$	$1\pm1^{(ab)}$

In unburnt treatment, there are no significant differences between populations. In the burned treatment there are differences for shrubs (χ2=11.69; p-value<0.05), native cover (χ2=8.36; p-value<0.05) and for total coverage (χ2=11.78; p-value<0.05).

There are significant differences between treatments for the population of Mira for native cover (χ2=7.62; p-value<0.05). In Tocha there are significant differences for other trees (χ2=3.57; p-value<0.05) and native cover (χ2=7.46; p-value<0.05). In Leiria there are significant differences for: shrubs (χ2=7.49; p-value<0.05), native cover (χ2=7.46; p-value<0.05) and total vegetation cover (χ2=7.48; p-value<0.05).

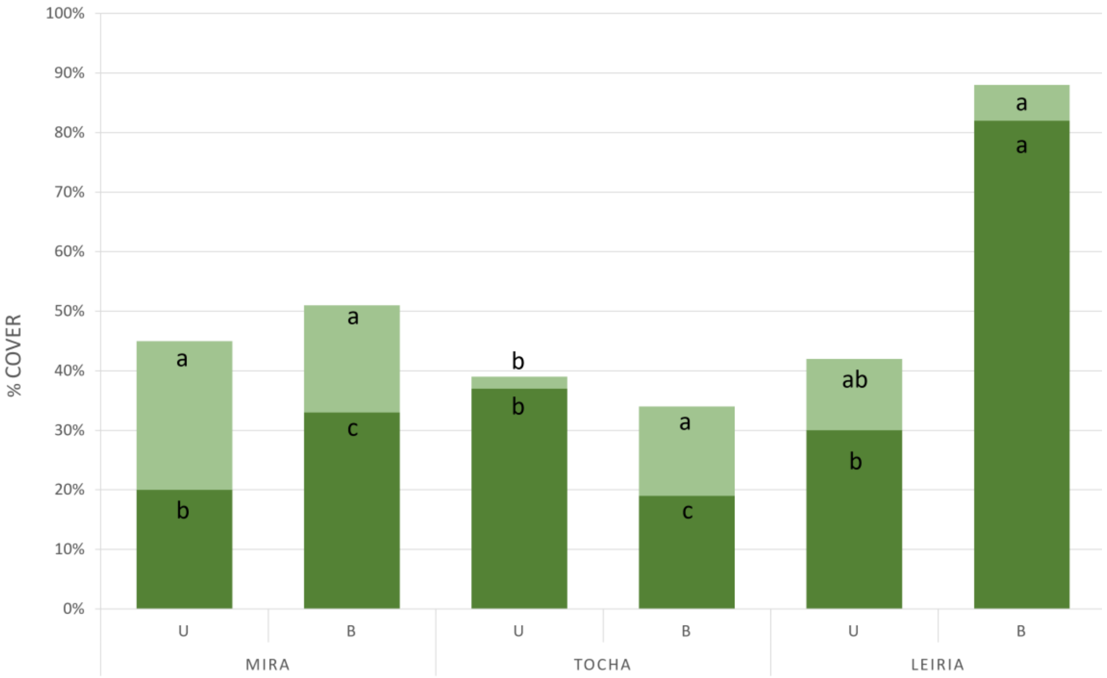


*Figure 3. Vegetation cover (%) by native and invasive on the three locations- Mira, Tocha and Leiria – in unburnt (U) and burnt (B) treatments. Native cover (**dark green**), Invasive cover (**Light green**). Bars represent the mean, and the different letter indicates significant differences (p<0.05).*

4.3.2 Invasive cover

The most invaded site in the two treatments is Mira (26% unburnt, 17% burnt). In unburnt the least invaded place is the Tocha (1%) and in burnt the least invaded is Leiria (7%) (Table 6). Except for the Tocha, the unburnt treatment has a higher average invasive coverage than the burned one.

Invasive vegetation cover was divided into two categories, in which the percentage of coverage (invasive herbaceous and invasive trees) were measured. Analyzing by type of invasive cover, tree invaders have greater expression. As for invasive herbaceous in unburnt treatment, the site with the highest average coverage is Mira (6%), the other two sites do not have invasive herbaceous in this treatment. For the burnt treatment, these have a higher expression in Mira (12%) and lower in Leiria (4%). For invasive trees in unburnt treatment, the location with the highest coverage is Mira (20%) and the lowest is in the Tocha (1%). In the burnt treatment, the place with the highest coverage is the Tocha (7%) and the lowest in Leiria (3%) (Figure 5).

Table 6. Invasive vegetation cover characterization (mean±sd) by typology on the three locations- Mira, Tocha and Leiria – in unburnt (U) and burnt (B) treatments. The different letter indicates significant differences (p<0.05).

	Invasive cover (%)					
	Mira		**Tocha**		**Leiria**	
	U	**B**	**U**	**B**	**U**	**B**
Total Invasive cover	$26\pm20^{(a)}$	$17\pm11^{(a)}$	$1\pm1^{(b)}$	$15\pm13^{(a)}$	$10\pm8^{(ab)}$	$7\pm3^{(a)}$
Invasive Herbaceous	$6\pm10^{(b)}$	$12\pm6^{(ab)}$	$0\pm0^{(b)}$	$8\pm8^{(a)}$	$0\pm0\%^{(b)}$	$4\pm4^{(ab)}$
Invasive Trees	$20\pm13^{(b)}$	$5\pm7^{(a)}$	$1\pm1^{(c)}$	$7\pm6\%^{(a)}$	$10\pm7\%^{(bc)}$	$3\pm2^{(a)}$

For the unburnt treatment there are significant differences between populations for invasive trees (χ2=5.79; p-value<0.05) and for total invasive cover (χ2=5.66; p-value<0.05). In the burnt treatment there are no differences between populations. In Mira there are no significant differences between treatments. In Tocha there are significant differences for invasive herbaceous cover (F=5.37; p-value<0.05) and for total invasive cover (χ2=6.40; p-value<0.05. In Leiria there are significant differences for invasive arboreous cover (χ2=3.85; p-value<0.05).

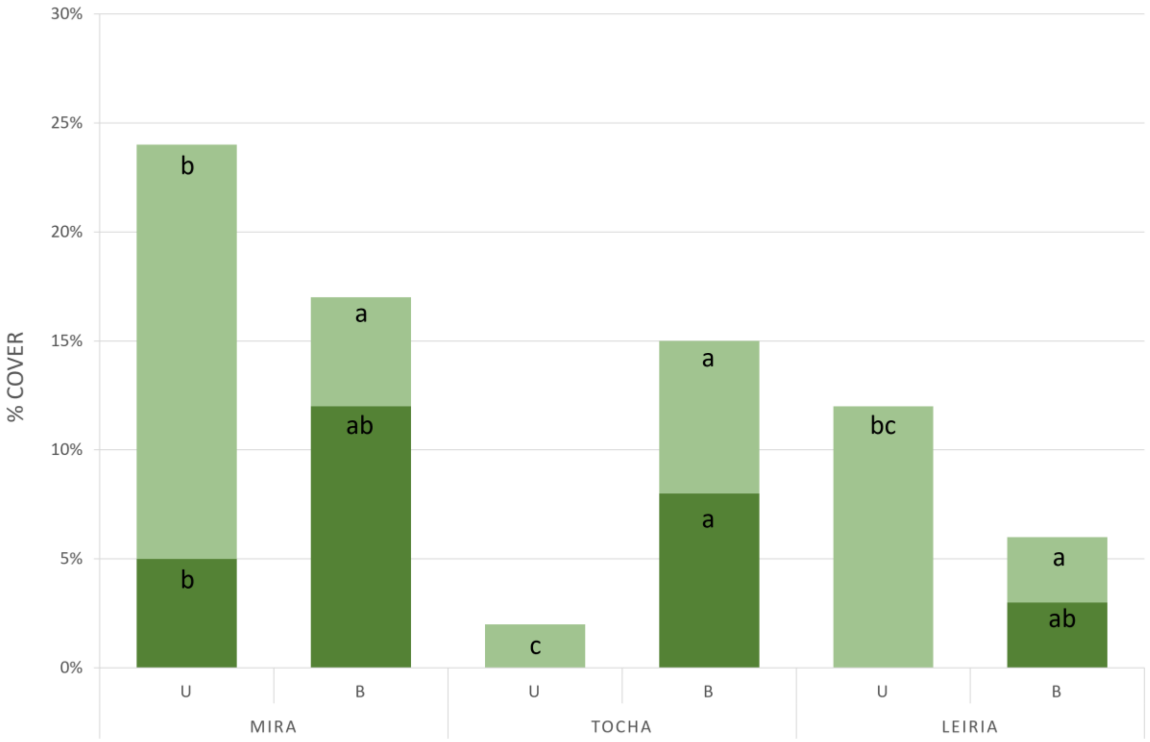


*Figure 5. Invasive vegetation cover (%) by typology on the three locations- Mira, Tocha and Leiria – in unburnt (U) and burnt (B) treatments. Invasive herbaceous (**dark green**), Invasive trees (**light green**) Bars represent the mean, and the different letter indicates significant differences (p<0.05).*

4.3.3 Vegetation Height

Regarding the total heights of the cover, these are for the top three populations in unburnt treatment. In this treatment, the place with the highest average heights of cover is Mira (132.5 cm) and less in Leiria (51.4 cm) (Table 7). In the burned treatment, the opposite is true, with Leiria being the place where heights are greatest (46.3 cm) and Mira where they are lowest (32.9 cm). Analyzing by type of cover (native vs invader), for the native cover in the unburnt treatment the highest height is in Mira (113.3 cm) and the smallest in Leiria (52.8 cm). Still for the native cover, but in the burned treatment, the opposite is true, the highest average height is in Leiria (55.3 cm) and the lowest in Mira (35.2 cm). As for the invasive cover, in the unburnt treatment, the highest average cover height is in Mira (120 cm) and the lowest in Leiria (50 cm), following the same pattern as the native cover (Figure 6). In the burned treatment, the place with the highest average height is the Tocha (32.9 cm) and the smallest in Leiria (27.5 cm).

Table 7. Medium height of vegetal cover (mean±sd) on the three locations- Mira, Tocha and Leiria – in unburnt (U) and burnt (B) treatments. The different letter indicates significant differences (p<0.05).

	Medium heigth (cm)					
	Mira		**Tocha**		**Leiria**	
	U	**B**	**U**	**B**	**U**	**B**
Native cover	113.3±12.5(b)	35.2±8.6(a)	60.4±37.4(ab)	45.1±14(a)	52.8±30.5(a)	55.3±20(a)
Invasive cover	120±157.8(ab)	29.5±16.9(a)	67.5±75.4(ab)	32.9±11.8(a)	50±15.4(b)	27.5±16.2(a)
Total cover	132.5±65,1(b)	32.9±14.3(a)	64.4±48.3(ab)	38.8±11.5(a)	51.4±20.6(a)	46.3±25.5(a)

As for the heights of the cover for unburnt treatment there are significant differences between populations for the height of the native cover (χ2= 4.44; p-value<0.05) and for the height of cover in general (χ2= 4.89; p-value<0.05). When analyzing the results by population, there are significant differences between treatments for the population of Mira native cover height (χ2= 4.15; p-value<0.05) and total cover (χ2= 5.40; p-value<0.05). In Tocha there no significant differences. In Leiria there are for the heigth of invasive cover (χ2= 5.11; p-value<0.05).

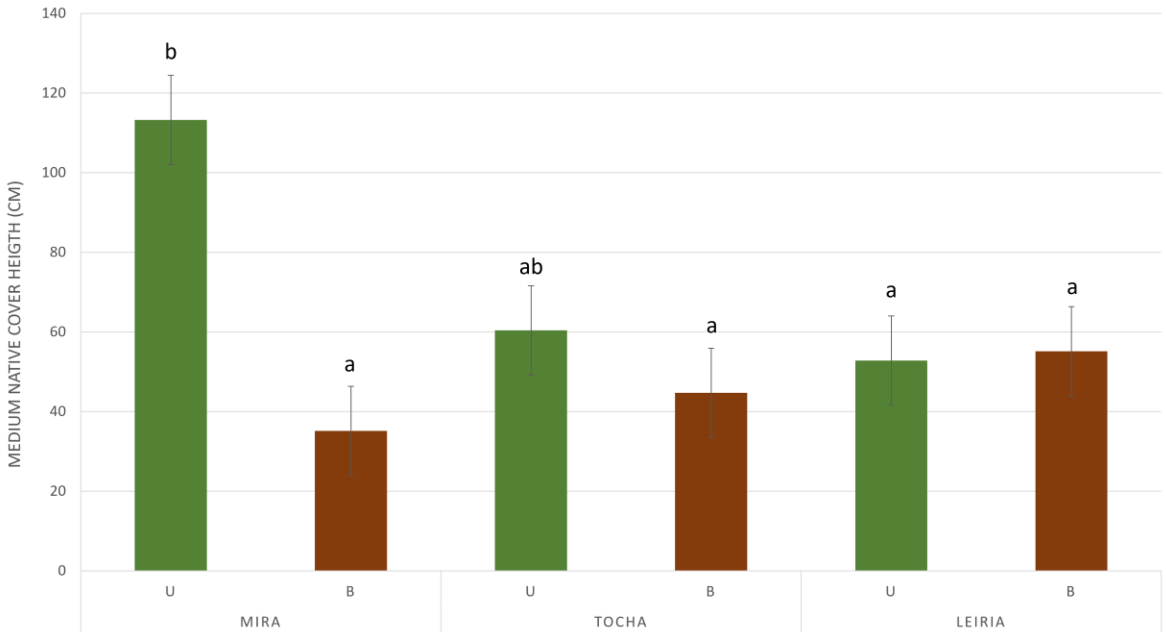


Figure 6. Medium height of native cover on the three locations- Mira, Tocha and Leiria – in unburnt (U) and burnt (B) treatments. ***.*** *Bars represent the mean + error and the different letter indicates significant differences (p<0.05).*

4.4 Regeneration of *Pinus pinaster*

4.4.1 Survival

Pinus pinaster regeneration varied between populations and treatments, concerning seed germinations and seedling survival. Mira had the lower survival value, for both the unburnt treatment (46%) and for the burnt treatment (80.5%) (Figure 7). The place where survival was higher in both treatments was Leiria (97.2% in unburnt, 98.7% in burnt). For the three sites, survival is always greater in the burned treatment than in the unburnt. There are significant differences between all populations in the unburnt treatment (χ2= 27.64; p-value<0.05). There are significant differences for survival between treatments in Mira (χ2= 11.13 p-value<0.05).

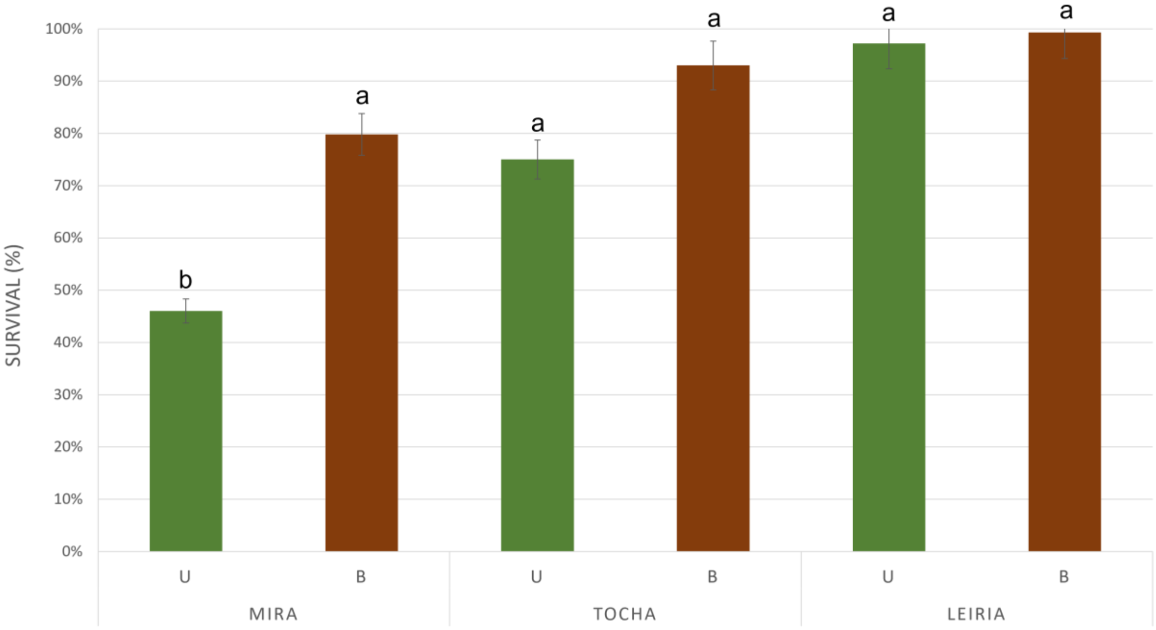


Figure 7. Pinus pinaster regeneration medium survival of Mira, Tocha and Leiria populations in unburnt (U) and burnt (B) treatments. Bars represent the mean +error and different letter indicate significant differences (p<0.05).

4.4.2 Regeneration density

Mira is the place that has the highest regeneration density both in the control (6.6 m^2) and for the burnt (1.5 m^2) (Figure 8). It appears that for the three sites, the density of regeneration was always greater in the unburnt treatment, with this difference being more contrasting in Mira. Leiria is the place with the lowest medium regeneration in the unburnt treatment (2.6 m^2) and Tocha in the burnt treatment (0.75 m^2). There are no differences in density of living regeneration between populations for both burnt and unburnt treatment. Among treatments, there are only differences of relevant live regeneration in the Tocha ($\chi 2 = 6.61$; p-value<0.05).

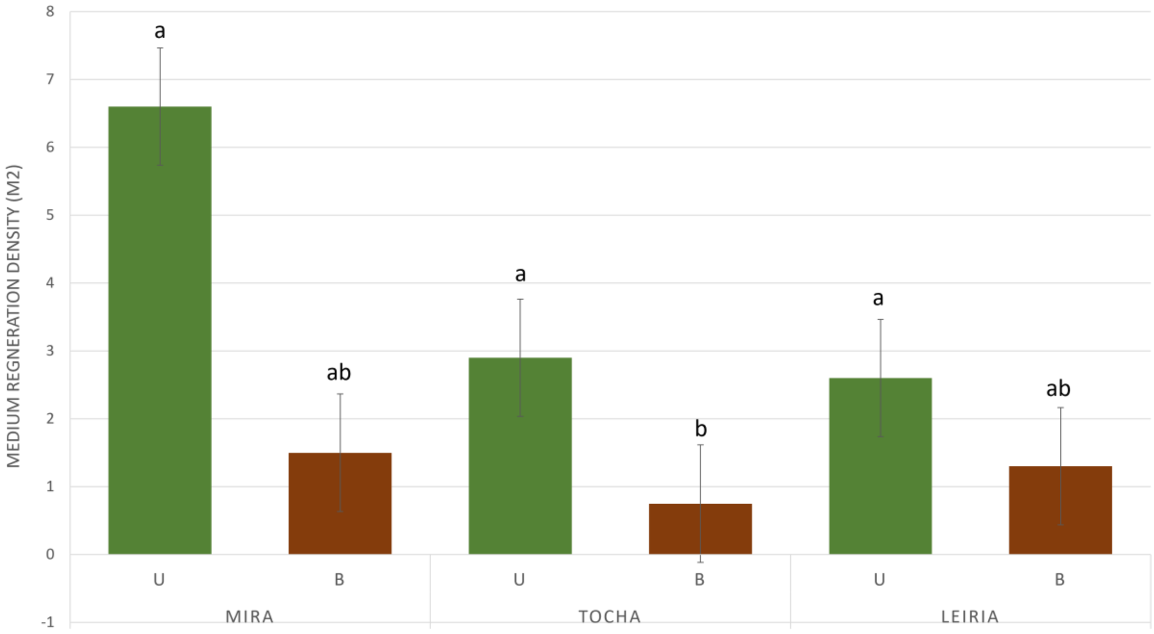


Figure 8. Pinus pinaster regeneration medium density of Mira, Tocha and Leiria populations in unburnt (U) and burnt (B) treatments. Bars represent the mean +error and different letters indicate significant differences (p<0.05).

4.4.3 Regeneration age

Tocha is the only unburnt location that has regeneration older than 1 year, although only 1% of 2-year-old pines (Figure 9). In the remaining locations, only pine trees aged up to 1 year were found; 73 in Mira and 62 in Leiria. In the burned treatment, pine trees were registered until the age of three. Tocha presents for this treatment 20 pine trees with one year old and 12 with two years old. Tocha features 14 with 1-year-old and 3 with 2-year-old. In Leiria, pine trees of three ages, 11 of one year, 9 of two years, and 5 of three years were registered. There are significant differences between Tocha and other populations in the unburnt treatment (F= 5.14 p-value<0.05). In analyzing the differences between treatments, only Mira registers significant age differences (F=15.35; p-value<0.05).

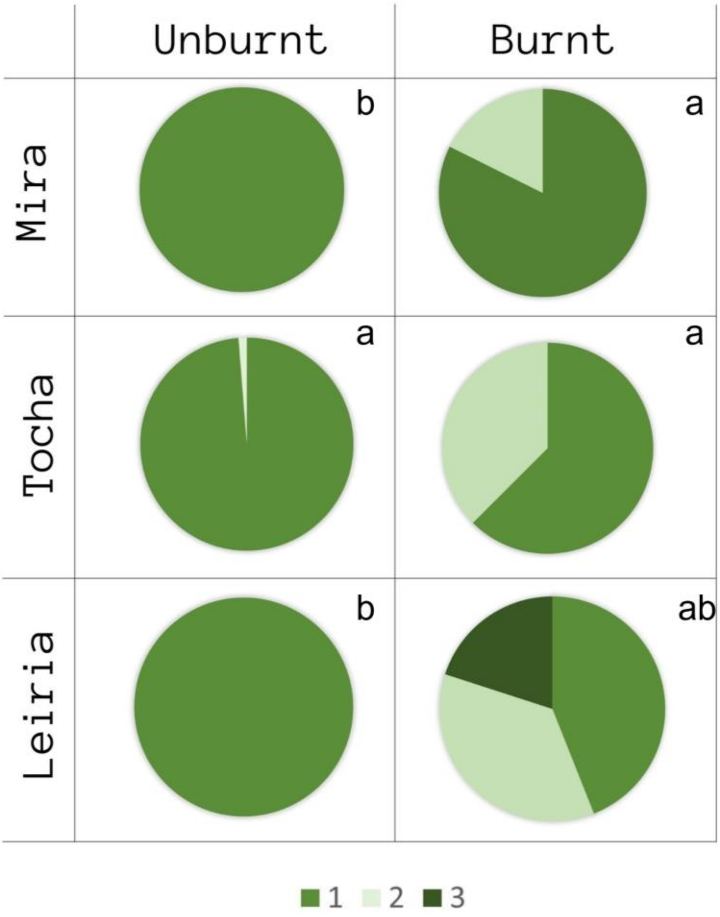


Figure 9. Pinus pinaster age by classes (1 year, 2 years, 3 years) in Mira, Tocha and Leiria populations for unburnt and burnt treatments. Different letters indicate significant differences (p<0.05).

4.4.4 Regeneration heigth

In the burnt treatment the heights for the three age classes are higher than those of the unburnt treatment (Figure 10). For the one-year-old class in unburnt treatment, the place with the highest height is Leiria (5.1 cm), in the treatment for the same class the highest average height is found in the Tocha (6.1 cm) (Table 8). For the 2-year-old class, in unburnt only Tocha registers seedlings with an average height of 6 cm, whereas in the burnt treatment the place with the highest average height is Leiria with 31.6 and the lowest height in the Tocha (10 cm). The only place that records 3-year-old pines is Leiria, in the burned treatment that has an average height of 70 cm. There are significant differences in regeneration height between populations for in the burnt treatment (F=5.19; p-value<0.05). There are significant differences between the height of regeneration between treatments in Mira (χ2= 11.57; p-value<0.05), Tocha (F=7.03; p-value<0.05) and Leiria (F= 11.57; p-value<0.05).

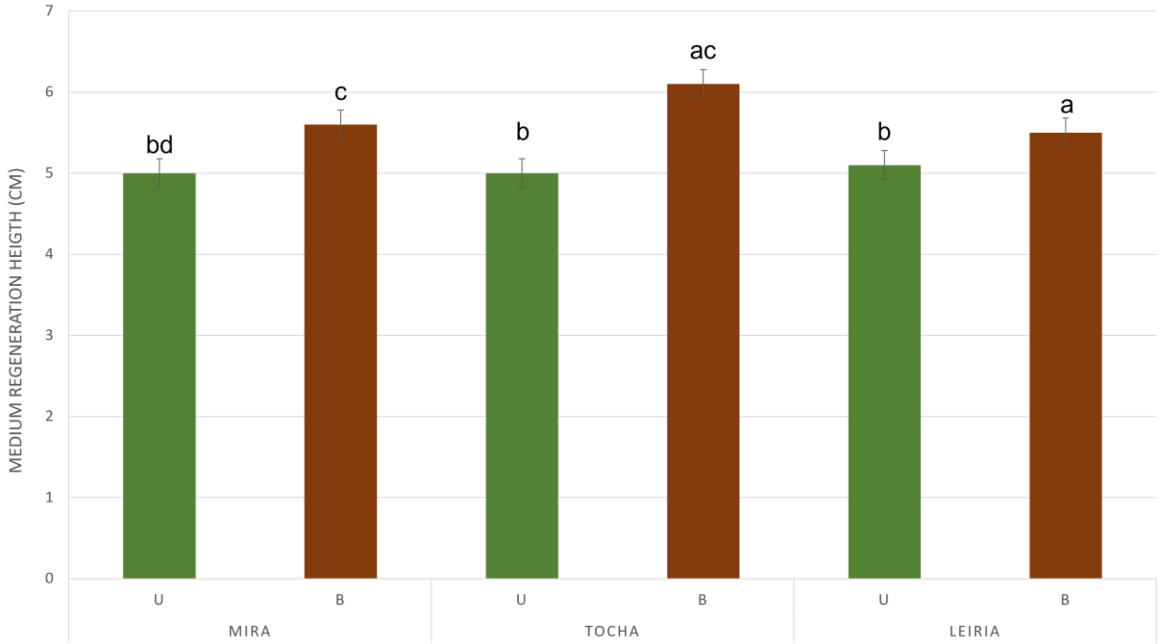


Figure 10. Pinus pinaster regeneration medium height of Mira, Tocha and Leiria populations in unburnt (U) and burnt (B) treatments. Bars represent the mean+error and different letters indicate significant differences (p<0.05).

Table 8. Pinus pinaster regeneration characterization (mean±sd) on the three locations- Mira, Tocha and Leiria – in unburnt (U) and burnt (B) treatments. The different letter indicates significant differences (p<0.05).

	Regeneration											
	Medium age (years)		**Medium Density (Pine/m2)**		**Medium Survival (%)**		**Medium heigth by age class (cm)**					
							1 year		**2 year**		**3 Year**	
	U	**B**	**U**	**B**	**U**	**B**	**U**	**B**	**U**	**B**	**U**	**B**
Mira	1±0$^{(bd)}$	1.3±0.2$^{(c)}$	6.6±7.1$^{(a)}$	1.5±1.1$^{(ab)}$	46±25.0$^{(b)}$	70.5±19.2$^{(a)}$	5	5.6	6	1.6	-	-
Tocha	1±0$^{(b)}$	1.2±0.4$^{(ac)}$	2.9±1.3$^{(a)}$	0.75±0.72$^{(b)}$	75.1±30.8$^{(a)}$	93±7.3$^{(a)}$	5	6,1	-	10	-	-
Leiria	1±0$^{(b)}$	1.7±0.9$^{(a)}$	2.6±1.5$^{(a)}$	1,3±2,1$^{(ab)}$	97.2±6.9$^{(a)}$	98.7±49.2$^{(a)}$	5.1	5.5	-	31.6	-	70

4.4.5 Pinecone

Tocha has the highest mean values of pinecones both in unburnt treatment (1.6 p/m^2) (Table 9) and in burnt (1.3 p/m^2). Regarding the lowest average values, in the unburnt treatment they are in Leiria (0.5 p/m^2) and in the unburnt treatment they are in Mira (0.2 p/m^2), which is the lowest recorded value.

There are significant differences for the number of pines in the unburnt treatment (F= 5.18; p-value<0.05) and in the burnt treatment (χ2=4.96; p-value<0.05). There are significant differences between treatments only in Mira (χ2= 12.28; p-value<0.05).

Table 9. Pinecone density (mean±sd) on the three locations- Mira, Tocha and Leiria – in unburnt (U) and burnt (B) treatments. The different letter indicates significant differences (p<0.05).

	Average pinecone (m^2)	
	U	**B**
Mira	1.1±0.9$^{(abc)}$	0.2±0.7$^{(c)}$
Tocha	1.6±1.5$^{(ab)}$	1.3±2.3$^{(a)}$
Leiria	0.5±0.7$^{(abc)}$	0.3±0.4$^{(bc)}$

4.5. Factors influencing regeneration

4.5.1 Unburnt pine forest

In the unburned treatment, for the relationships between the biotic response variables and abiotic variables only correlations were found for regeneration survival (Table 10). This correlation was negative with maximum temperature of the first year after fire (Rho= -0.619; p-value<0.05); average precipitation of the first year (Rho= -0.566; p-value<0.05) and maximum temperature of the second year after fire (Rho=-0.703; p-value<0.05). Regeneration survival also showed positive correlations with height above sea level (Rho= 0.785; p-value<0.05) (Figure 11); mean temperature of the first year (Rho=0.678; p-value<0.05); minimum temperature of the second year (Rho=0.718; p-value<0.05); consecutive days without rain of the first year (Rho=0.577; p-value<0.05); minimum temperature of the second year (Rho=0.743; p-value<0.05) and consecutive days without rain of the second year (Rho=0.577; p-value<0.05). There was also a trend of negative relationship between regeneration survival and average rainfall of the second year.

*Table 10. Spearman's correlations between the biotic response variable and the abiotic variables for the unburnt treatment. Tx1= Medium temperature 1st year; Tmax1= Maximum temperature 1st year; Tmin1= Minimum temperature 1st; Px1= Medium precipitacion 1st year; DwR1 = Consecutive days without raning in the 1st year; Tx2= Medium temperature 2nt year; Tmax2= Maximum temperature 2nd year; Tmin2= Minimum temperature 2nd; Px2= Medium precipitacion 2nd year; DwR2 = Consecutive days without raning in the 2nd year. Positive correlations (**Green**), negative correlations (**Brown**) and trends (**Black**). P-values lower than 0.05 are marked with (*), p-values lower than 0.01 marked with (**) and non-significant p-values are marked with (n.s)*

	Topography and climate										
	Height Above sea (m)	**Tx1**	**Tmax1**	**Tmin1**	**Px1**	**DwR1**	**Tx2**	**Tmax2**	**Tmin2**	**DwR2**	**Px2**
Regeneration Density	-	-	-	-	-	-	-	-	-	-	
Regeneration Survival	0,785**	0,678**	-0,619**	0,718**	-0,566**	0,577**	-0,533*	-0,703**	0,743**	0,577**	-0,422(n.s)
Height of Regeneration	-	-	-	-	-	-	-	-		-	-

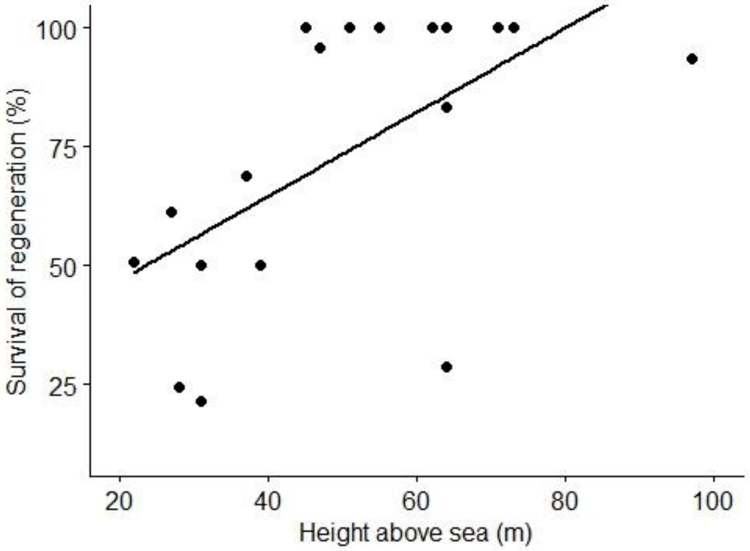


Figure 11. Graphic representation for Spearman's correlation between Height above sea (m) and the survival of the regeneration (%), for unburnt treatment

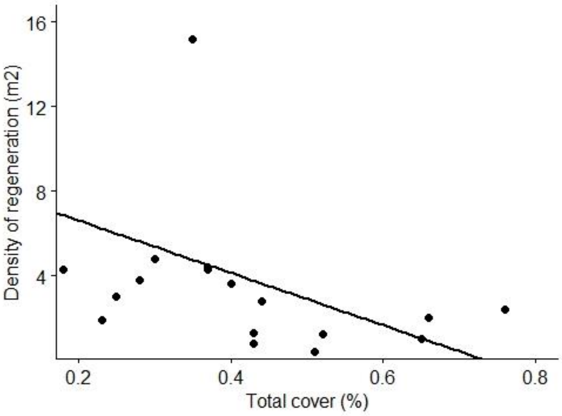
In the unburned treatment, for the relationships between biotic variables, we have for regeneration density a negative correlation with total cover (Rho=0.654; p-value<0.05) (Figure 12a), a negative trend with invasive tree cover and a positive trend with adult tree density (Table 11).

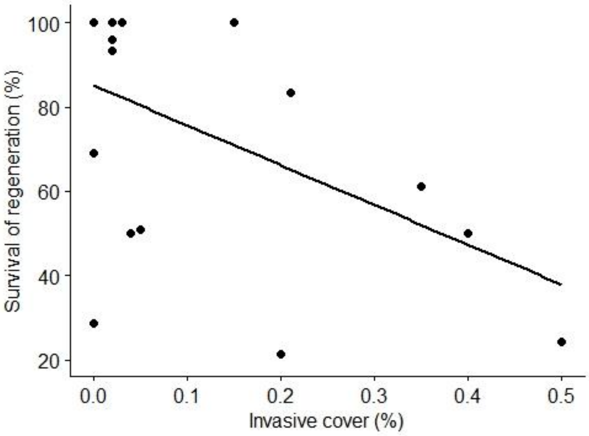
Regeneration survival showed a negative correlation with invasive herbaceous cover (Rho=-0.469; p-value<0.05), invasive cover (Rho=-0.560; p-value<0.05) (Figure 12b), and mean height of total cover (Rho=-0.622; p-value<0.05) (Figure 12c).

Regeneration height only showed a positive correlation with the average height of total cover (Rho=0.785; p-value<0.05) and a positive trend with the average height of the native cover.

*Table 11. Spearman's correlations between the biotic response variable and the biotic variables for the unburnt treatment. Positive correlations (**Green**), negative correlations (**Brown**) and trends (**Black**). P-values lower than 0.05 are marked with (*), p-values low lower than 0.01 marked with (**) and non-significant p-values are marked with (n.s)*

	Sttlement	**Vegetal cover**					
	Tree density	**Invasive herbaceous**	**Invasive trees**	**Invasive cover**	**Height of native cover**	**Total cover**	**Height of total cover**
Regeneration Density	0,454 (n.s)	-	-0,564(n.s)	-	-	-0,654*	-
Regeneration Survival	-	-0,469*	-	-0,560*	-	-	-0,622**
Height of Regeneration	-	-	-	-	0,485(n.s)	-	0,785**





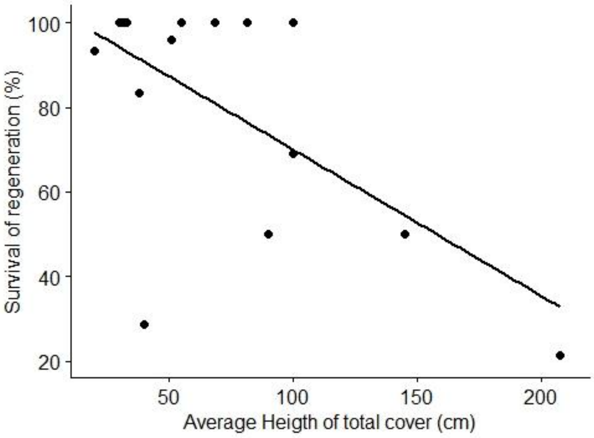


Figure 12. Graphic representation for Spearman's correlation between a) Density of regeneration (m2) and total cover (%); b) Survival of regeneration (%) and Invasive cover (%) and c) Survival of regeneration and Average height of total cover (%) for unburnt treatment

4.5.2 Burnt pine forest

In the burnt treatment regarding abiotic variables, density and regeneration did not show any correlation or trend (Table 12).

Regeneration survival showed negative correlations with distance from the sea (Rho= -0.539; p-value<0.05) (Figure 13a) and with second-year maximum temperature (Rho= -0.559; p-value<0.05). This variable presented positive correlations with the height above the sea (Rho=0.716) (Figure 13b) and with the minimum temperature of the second year (Rho=0.594; p-value<0.05). It also presented a tendency of positive relationship with the average temperature of the first year after the fire and a negative tendency with the maximum temperature of the first year.

Regarding the regeneration height, it showed a negative correlation with the maximum temperature of the first year (Rho= -0.530; p-value<0.05) and with the average precipitation of the second year (Rho= -0.580; p-value<0.05). This variable presented positive correlations with the average temperature of the first year (Rho = 0.678; p-value<0.05); a minimum temperature of the first year (Rho = 0.514); consecutive days without rain (Rho = 0.605; p-value<0.05) and with the minimum temperature of the second year (Rho = 0.588; p-value<0.05).

*Table 12. Spearman's correlations between the biotic response variable and the abiotic variables for the burnt treatment. Tx1= Medium temperature 1st year; Tmax1= Maximum temperature 1st year; Tmin1= Minimum temperature 1st; DwR1 = Consecutive days without raning in the 1st year; Tmax2= Maximum temperature 2nd year; Tmin2= Minimum temperature 2nd; Px2= Medium precipitacion 2nd year. Positive correlations (**Green**), negative correlations (**Brown**) and trends (**Black**). P-values lower than 0.05 are marked with (*), p-values lower than 0.01 are marked with (**) and the non-significant p-values are marked with (n.s).*

	Topography and climate								
	Distance to sea	**Height above sea**	**Tx1**	**Tmax1**	**Tmin1**	**DwR1**	**Tmax2**	**Tmin2**	**Px2**
Regeneration density	-	-	-	-	-	-	-	-	-
Regeneration survival	-0,539*	0,716**	0,448(n.s)	-0,501(n.s)	0,567*	-	-0,559*	0,594*	-
Heigth of Regeneration	-	-	0,679**	-0,530*	0,514*	0,605*	-	0,588*	-0,580*

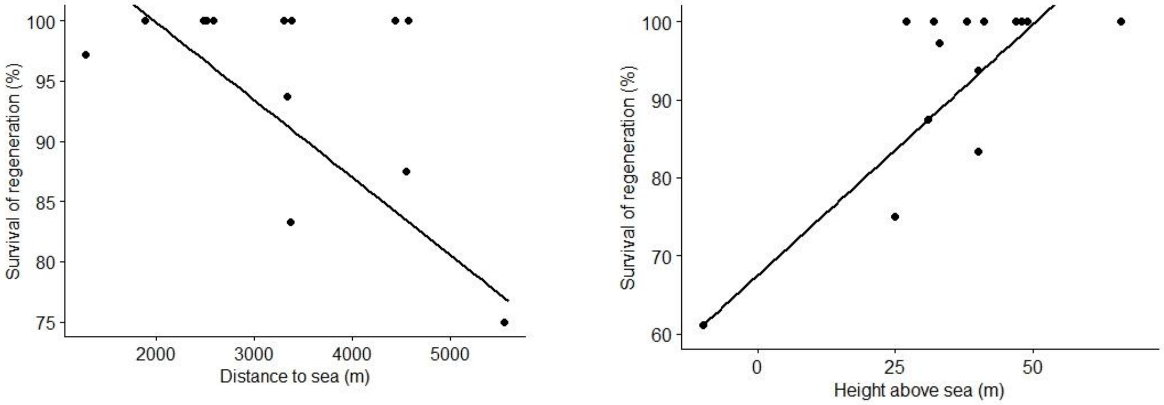


Figure 13. Graphic representation for Spearman's correlation between a) Survival of regeneration (%) and distance to sea (m) and) b) Survival of regeneration (%) and Height above sea (m) for burnt treatment

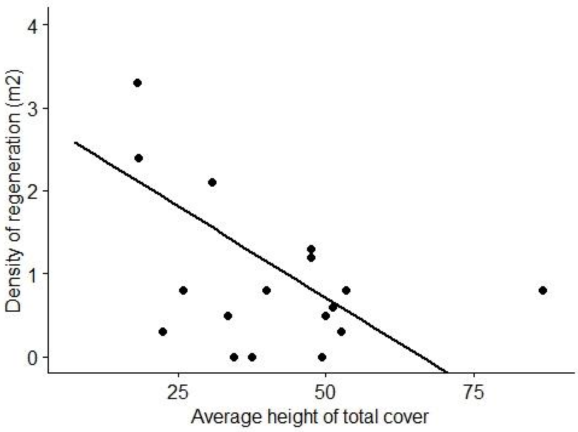
In the burned treatment the regeneration density showed negative correlations with the average height of the invasive cover (Rho=-0.619; p-value<0.05), the average height of the native cover (Rho= -0.566 p-value<0.05), and with the average height of the total cover (Rho= 0.562; p-value<0.05) (Table 13). A negative trend was still observed with the invasive tree cover.

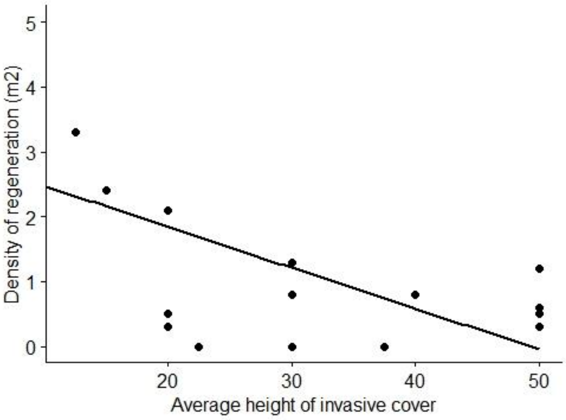
Regarding regeneration survival, it showed a negative correlation with the basal area (Rho= -0.814; p-value<0.05) and with herbaceous cover (Rho= -0.786; p-value<0.05) (Figure 14). There was a positive relationship between regeneration survival and shrub cover.

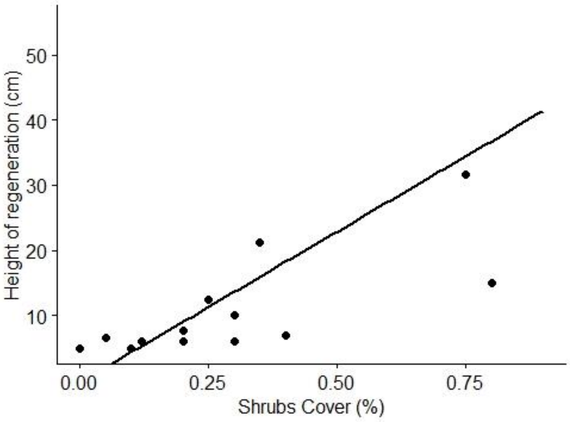
The height of regeneration showed a positive correlation with herbaceous ground cover (Rho= 0.737 p-value<0.05), with native cover (Rho= 0.717; p-value<0.05), and with total cover (Rho= 0.667; p-value<0.05). There were trends of a negative relationship between regeneration height and stand age and with litter cover on the ground.

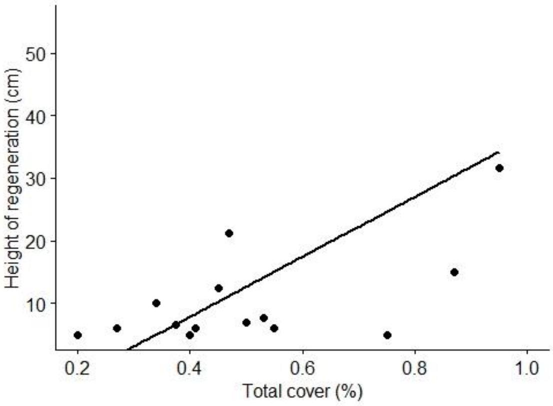
*Table 13. Spearman's correlations between the biotic response variable and the biotic variables for the burnt treatment. Positive correlations (**Green**), negative correlations (**Brown**) and trends (**Black**). P-values lower than 0.05 are marked with (*), p-values lower than 0.01 marked with (**) and non-significant p-values are marked with (n.s).*

	Stllement		Soil cover		Vegetation cover							
	Tree age	Basal area	Litter	Herbaceous that cover the soil	Invasive tree	Height of Invasive cover	Native cover	Height of Native cover	Total cover	Height of Total cover	Herbaceous	Shrubs
Regeneration density	-	-	-	-	-0,419(n.s)	-0,619**	-	-0,562*	-	-0,566*	-	-
Regeneration survival	-	-0,814**	-	-	-	-	-	-	-	-	-0,786**	0,459(n.s)
Height of Regeneration	-0,555(n.s)	-	-0,479(n.s)	0,737**	-	-	0,717**	-	0,667**	-	-	0,799**









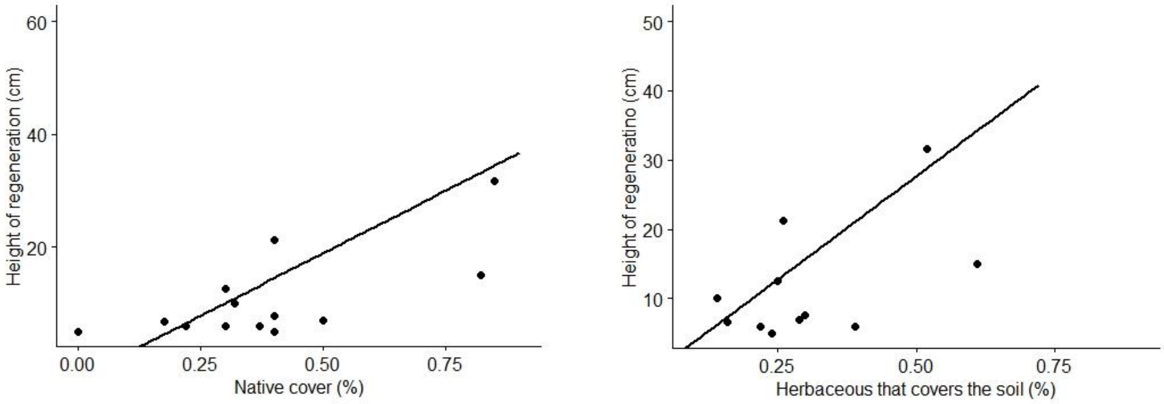


Figure 14. Graphic representation for Spearman's correlation between a) Density of regeneration (m2) and Average height of total cover (%); b) Density of regeneration (m2) and Average height of invasive cover (%); c) Height of regeneration (cm) and shrubs cover (%); d) Height of regeneration (cm) and total cover (%); e) Height of regeneration and Native cover (%) and f) Height of regeneration and Herbaceous that covers the soil (%).

5. Discussion

5.1 Topography

The topography conditions the planting, flat and ventilated lands that favor natural irrigation with altitudes up to 400m are the ideal conditions for *Pinus pinaster* stands (Centro Pinus, 1999). As for height above sea, this was shown to be positively correlated with the survival of regeneration for both treatments (Rho= 0.785, in unburnt; Rho= 0716 in burnt; p-value<0.05). Mira is the site for the two treatments with the lowest survival rates and is also the site with the lowest elevations, having significant differences from the other two sites for the two treatments. Despite the significant differences in height between populations in the two treatments, these are not ecologically relevant to justify the differences in survival of regeneration through the fact that at lower elevations, rising temperatures should magnify *P. pinaster* summer drought stress, growth, and survival decline, as explained by some authors who studied the effects of altitude in the production of *P. pinaster* forests (Candel-Pérez *et al.,* 2012; Häusser *et al.*, 2019). The topography/survival or topography/survival relationships have been mainly invested in high altitude populations. The relation between heigth above sea and the survival of regeneration may be related to the fluxes of the water, terraced slopes with flats areas provides a favors conditions por pine germination and establishment in the Mediterranean conditions (Pausas *et al.,* 2004).

The distance to the sea has a negative correlation with the survival of regeneration in the burnt treatment (Rho= -0.539; p-value<0.05) and the distance to the sea, is positively correlated with the density of adult trees (Rho= 0.521; p-value<0.05). Something that would be expected, as according to (Kumar *et al.,* 2012) w0ere regeneration was significantly affected by distance to sea due salt stress. The soil salinity is one of the main abiotic stresses, which restrict the plant survival and growth (Hameed *et al.,* 2013), although I cannot say that this relationship is due to the increase in salinity, as no soil analyses have been carried out.

5.2 Climate

Although there are no significant differences, the first year, concerning the second, had a higher average annual precipitation and a lower mean annual temperature for the three locations. In contrast has a higher maximum temperatures and minor minimum temperatures, and more frequent than in the second year. Also, it had longer drier periods that coincided with higher and more recurrent maximum temperatures.

The regeneration processes are stochastic phenomena within the dynamics of the forest (Paluch, 2005), they vary from one year to the next depending on the interactions between environmental parameters and the ecological interactions (Rodríguez García *et al.,* 2007), so differences in temperature and precipitation can be decisive for successful regeneration.

Through the result of the positive correlations between the average temperature of the first year and the survival of regeneration (Rho=0.679 p-value<0.05) in unburnt, the height of regeneration in the burnt treatment follows the same trend, with a positive correlation with the average temperature of the first year (Rho=0.679; p-value<0.05), which is lower than that of the second year. We can infer milder temperatures tend to act positively on regeneration (Matías *et al.*, 2017).

Through the number of consecutive days without rain, the shorter the drier period, the better the survival of the seedlings in the unburnt treatment (Rho= 0.577; p-value<0.05). The second year, with lower rainfall averages, shows a negative relationship with the survival of regeneration in burned (Rho= -0.589; p-value<0.05), reinforcing the role of water stress in the success of regeneration. In studies by (Matías *et al.,* 2017), the results agreed with those presented. In this, we tested the effect of increasing temperature and drought on the survival of regeneration in several species of the genus *Pinus* in a Mediterranean ecosystem and obtained lower survival rates when in warmer and dyer combinations. This was explained by the authors through root allocation and water stress, which translate into lower survival and growth rates. According to (Rodríguez-García *et al.,* 2011), the percentage of precipitation and the months in which it occurs are directly related to the success of regeneration. It was also demonstrated by (Ruano *et al.,* 2009) that summer rains have a significant influence both on germination and on the density of viable seedlings, which corroborates the fact that both germination and the survival of regeneration in the first year may have been compromised by climatic issues of water stress. With increasing temperatures and dry periods, regeneration is more dependent on spring precipitation to survive and grow (Férriz *et al.,* 2021). Apparently, freezing did not affect germination or seedling survival, as the negative temperatures recorded were of -1ºC during the February 2018 and -0.5 in the same month in 2019 at Mira. In coastal areas, due to the stability of temperatures, this phenomenon has little influence on regeneration.

5.3 Stand

The stands under study are mature stands (greater than 35) except for burnt treatments which, due to the heterogeneity of ages, have lower mean ages.

The acceptable tree density is between 1000 and 2222 tree/ha, depending on the compass and the productivity of the land (Centro Pinus, 2019). All sites have acceptable tree densities for production forests within the stipulated except for Leiria in the unburnt treatment which registered a density of 770 tree/ha.

The significant differences in DBH between treatments in the three locations are a result of this heterogeneity of ages, with the mean age and DBH being lower in burn treatments.

Is to expect that the tree stand may to affect the seedling and sapling in different ways: the avaibility of resources (light, water, nutrients) and in other factors like temperature of the air, temperature of the soil and the microbiotical activity (Lundqvist and Fridman, 1996). In unburnt treatment we only found a tendency of a density of trees are positive related to density of the regeneration, this can be explained by the biggest area of canopy may correspond a big canopy seed bank (Pausas *et al.*, 2004). In post-fire areas we have different results with a negative correlation between the pine biomass, measured like basal area, and the survival of regeneration. These results are contrary to those expected, since, for the reasons explained above, in studies on burnt areas (Pausas *et al.,* 2004) demonstrated a positive correlation between biomass production and regeneration. Also, exists a negative relation between the heigth of regeneration and the tree age. This negative relation can be explained by the effects of competition by resources, (Comita and Hubbel, 2009) studies shows that basal area, tree density or big crown projection can be provides a negative effect on the seedling survival and growth. *Pinus pinaster* has a tolerance to shadow for germination but with the increasing of age, in sapling stage have an increase of demand of light, being intolerant to shade (Fernandes and Guiomar, 2007).

5.4 Soil cover

When comparing the herbaceous coverage that covers the soil, we find that there is a greater coverage in the burned treatment than in unburnt, except in the case of Tocha, where the coverage is very similar in both conditions (16% in unburnt, 15% in burnt). The same for Bare soil, with bigger representativity in burned than in unburnt. The opposite is true of the presence of litter.

Analyzing the coverage in its components there is a significant positive correlation between herbaceous that cover the soil and the heigth of regeneration (Rho= 0.737) and there is only the tendency of the litter coverage to negatively influence the survival of regeneration. This is explained by (Guerra and Bravo, 2004) who point out the barrier effect that the litter constitutes, preventing the contact of the seed with the soil, germination, growth, and survival. This results are corroborate the by (Rodríguez García *et al.*, 2007), showing that the soil cover decreases the probability of obtaining at least 2000 seedlings per hectare. It is observed that, in burned pine forest there is greater coverage of herbaceous soil, that is positively correlated with regeneration, this can be explained by phenomena of facilitation, the herbaceous protect the young pines of the temperature, keep the soil moisture (Gómez-Aparicio *et al.*, 2005).

5.5 Plant cover

The interpretation of the effects of vegetation cover depends on the regime of crowns in question - open canopy (post-fire) or closed canopy (unburnt forest) - as this will determine whether we are under the influence of facilitation or competition processes (Rodríguez- García *et al.,* 2011). According to (Bertness and Callaway, 1994), in unproductive environments such as those studied, the phenomenon of facilitation overlaps with the phenomena of competition, and this is verified in the burned environment.

In the burnt treatment we have positive correlation between the height of regeneration and the native cover (Rho: 0.717); the total cover (Rho: 0.667) and a trend to positive relation between the survival of regeneration and the shrubs cover. Demonstrating the positive influence of native cover on survival and regeneration growth, which can be explained by studies of (Gómez-Aparicio *et al.,* 2004, 2005) who found that the cover increased the survival and height of seedlings of plants of the genus *Quercus* and *Pinus*. This can be explained through phenomena that facilitates shrub species on pine seedlings. This facilitating process can occur through the canopy effect, through chemical/physical changes in the soil, or protection against herbivory (Gómez-Aparicio *et al.,* 2005). The canopy effect, protects the regeneration of the radiation and lowers the air and soil temperature, avoiding losses due to evapotranspiration and maintaining the necessary humidity for development, since for small plants (less tolerant to stress) the availability of water can be more determinant than light or nutrients (Aguilera *et al.*, 1999; Gómez-Aparicio *et al.*, 2005; Rodríguez-García *et al.,* 2011). The shrubs effects increase the concentration of nutrients in the soil and the mycorrhizal associations for nitrogen fixation (Soraine, 1986). It is emphasized by (Gómez-Aparicio *et al.,* 2005) the increase in the concentration of potassium in the presence of shrubs, which contributes to the resistance to drought, through osmotic adjustments and reduction of perspiration rates, and enables more efficient use of water. The positive relations of shrubs and native cover in survival and height of regeneration in burnt treatment, shows that the canopy effect of native vegetal cover after a fire may be the key to the resistance of seedlings to summer droughts, with shading being the key facilitating mechanism for successful regeneration in Mediterranean ecosystems after a fire since its effect is superior to the soil effect (Gómez-Aparicio *et a*l., 2005). But in the other hand, we have a negative relation between the heigth of cover and the density of regeneration (rho=-0.562 for the native cover; rho=0.566 for total cover), these results show that exists a limit of heigth of the cover for this facilitation relation. It is also noteworthy that, except for Tocha, in all other places in burned pine forest there is greater coverage of shrubs and herbs, which confirms the natural succession.

In the unburnt treatment, in a closed canopy regime, when compared to the burnt treatment, this relationship changes and there is a negative correlation between the regeneration density and the total cover (Rho=-0.654). There is a negative correlation between the survival of regeneration and the average height of total cover (Rho=-0.622). This can be explained through a relationship of competition for resources, such as the availability of nutrients, light, and water. Although the pine tree is a species that tolerates shade in the first months of life, the plants lose their shade tolerance and rarely exceed the age of more than one year in a closed canopy regime, (Fernandes and Guiomar, 2017), which corroborates the data of ages recorded in unburnt pine forest.

5.6 Invasive cover

In burnt treatment, invasive cover is shown to have negative impacts on density and survival, of regeneration. The negative correlation between the heigth of invasive cover and the density of regeneration (Rho= -0.619), demonstrates the impact of these. There is also a trend of negative relationship between the density of regeneration and the cover of invasive trees. In the unburnt treatment, there is the same trend for invasive trees, to be negative for survival of regeneration. Also, the invasive cover has a negative relation with the regeneration survival (Rho=-0.560), and a negative relation between the survival of regeneration and cover of invasive herbaceous (Rho=-0.469). Thus, demonstrating that there is the competition between the regeneration of pine and the invading cover, especially the tree, made up of *Acacia longifolia*. This species causes major changes in the structure of the forest. In studies carried out by (Rascher *et al.,* 2011a; Rascher *et al.*, 2011b), on the Portuguese coast, it was possible to prove that the regeneration of native species (including *Pinus pinaster*), is negatively affected by the presence of *Acacia longifolia*. This can be explained: by the effect of this invasive on soil properties such as moisture, density, and water holding capacity (Segoli *et al.,* 2008; Marchante *et al.*, 2018) through changes in the nutrient cycle, for example, due to its high phosphorus consumption (Rothe and Binkley 2001); by changes in the structure of vegetation that cause microclimate differences and alter the light regime; and changes in the water cycle (Rasher *et al.,* 2011a). The dominant reason for the competition relationship between *P. pinaster* and *A. longifolia* is explained by the effect of the invader on the use of water and the rates of carbon fixation, leading to a decrease in the regeneration density and the size of the same. On the other hand, *Acacia longifolia* can have a facilitating effect on N2 fixation, which is cancelled out by the effect of competition for water and light (Rasher *et al*., 2011b). The *Acacia spp.* also modificies the accumulation of fuel changing the recurrence of fire and the fire regime, may not allow time for regeneration to establish itself until adulthood to ensure a new cohort (Marchante *et al.,* 2018).

According to the results, native species such as *camarinha* (*Corema album*), strawberry tree (*Arbustus unedo*) and gorse (*Ulex parviflorus*) (Centro Pinus, 2020), must be preserved in the management, and the invasive species must be cut in a selective manner, mainly the larger ones, such as, in the case, *Acacia dealbata*, the most common invasive tree in the three areas of study. The management of shrub species should not be carried out two or three years after the fire, for the benefit of establishing regeneration, through facilitation processes (Miller *et al.*, 1991). Around that age, due to the differentiation, the roots of plants and the separation of niches in the subsoil, ecological relations go from facilitating competition, being therefore beneficial for the development of trees, cutting, which allows larger trees to increase the heterogeneity of the population (Miller *et al.,* 1991).

5.7 Regeneration

In all locations the regeneration density is considered successful for production forests, since in the three locations for the two treatments there are values and density higher than 3000 seedling/ha (Guignabert *et al.*, 2020). Highlighting that on many plots, the pine densities observed will be reduced as the pines get bigger. Mira, despite having high regeneration densities, has the lowest survival rates, which may indicate difficulty in the establishment. This may be due to the high levels of invasion (25% in the control; 18% in burnt) and its composition is mostly invasive trees, thus having a greater coverage height (200 cm in unburnt and 39 cm in burnt). These two variables show a correlation or tendency to be negative for regeneration density and survival. In Leiria, on the other hand, we see the opposite behavior. With the lowest regeneration densities of the three sites, especially in burnt, it has very high survival rates, demonstrating that despite low germination, between 97-99% of the seedlings can survive the first year, reaching higher ages. This may due explain to the the low level of invasive cover (12% in unburnt, 6% in burnt), that have a negative tendency to survival in burnt treatment and the high cover of native species that have facilitating relations with regeneration. In the unbrunt treatment, despite high regeneration densities, the pines did not exceed, at the date, the age of one year, except for Mira, where only 1.2% of the sampled pines reached two years of age, demonstrating a difficulty in establishing. This is explained by the fact that in closed canopies the seedlings need to compete for light. In these areas unless management measures are taken to create clearings the regeneration will continue in one year cycles, not becoming sustainable in the long term as they will not reach ages of maturity that allow the creation of a new cohort (Gómez-Aparicio *et al.,* 2005).

6. Conclusions and future perspectives

This work provides insights into relationships between climate, vegetation cover and invasive cover with the success of establishment of regeneration of *Pinus pinaster*. Among the contributes of the present work I like to highlight the following questions: (i) climate and topography affect the success of regeneration, whereas soil cover shows no relationship; (ii) The success of regeneration is determined by competitive and facilitative relationships; (iii) Regeneration competes with invasive cover and higher cover heights and (iv) Regeneration benefits from the presence of native vegetation.

The regeneration density is no difference between the populations under study, having density values to become viable. The place that has the greatest care to consider due to the difficulty of establishment is Mira, where studies must be reinforced to understand if there is a need for densification of regeneration. The place with the most promising regeneration is Leiria, with high survival rates and heterogeneous ages of regeneration indicating a greater success. The variables that best explain the success of regeneration are the height of the cover, the percentage of invasive cover and the native cover. There are processes of facilitation and competition in the dynamics of natural regeneration, with green vegetation having a competitive role and after the fire a facilitating role. Management measures are suggested, such as cutting down invaders as both unburnt and burned damage the density, survival, and height of seedlings, and preserving native vegetation at least until 3 years after fire, in the burned areas through facilitation processes will contribute to greater survival and greater regeneration growth.

It would be important to carry out this study with a greater sampling effort, as it would be expected to do if there were no impediments caused by the pandemic COVID-19. I think it would have been interesting to include variables that were not possible for me to work on, such as the opening of the canopy, which is indicated in the literature as one of the success factors for regeneration; the availability of seeds, to confirm whether the differences in regeneration are caused by different seed densities or by biotic/abiotic conditions; the physical-chemical parameters of the soil; and the most effective assessments of flora, to understand the biological relationships between species. It would be interesting to proceed with laboratory work, to test the germination in the different substrates with controlled factors, to understand the influence of the soil cover typology in the germination and the establishment of the pines since through these results it was not clear enough.

There are several knowledge shortcomings in different areas, such as the state of regeneration of national forests; the availability of seed in national forests; the effects of soil cover; and the competition/facilitation processes in the development of the plant and mycorrhizal relations in the establishment. It is necessary to create study plots where different models of silviculture can be tested in different habitats, so that silviculture measures more adapted to each type of disturbance of different forest ecosystems can be established. It is also necessary to increase the areas of seed production for a greater representation of some regions of provenance so that in the event of a fire, seeds can be chosen that guarantee greater success of establishment and production. It would also be of interest to map settlements more susceptible to disturbances such as fires and storms, as well as the state of regeneration existing in Portuguese pine forests.

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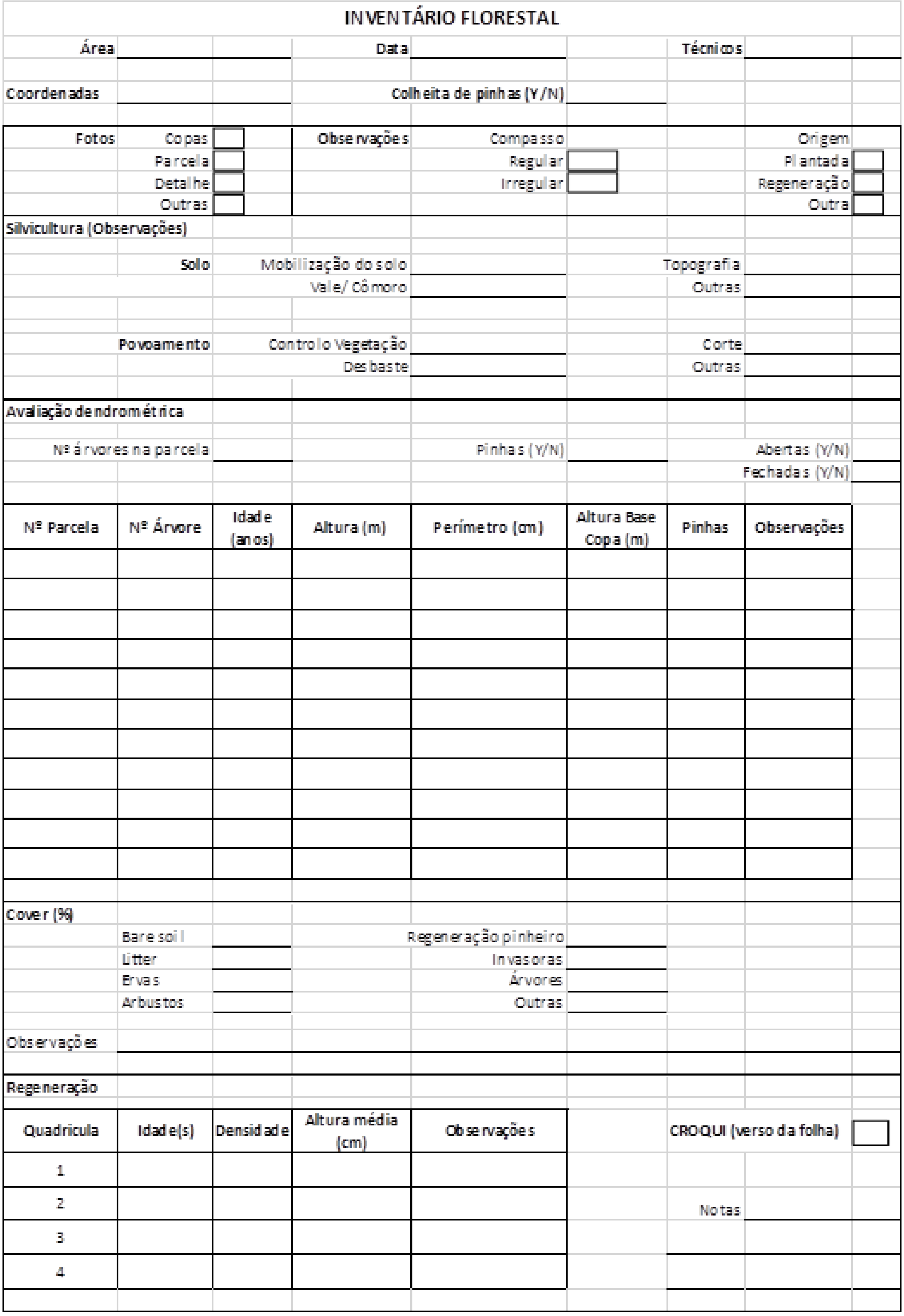
8. Suplemmentary material

Supplementary Material 1- Field sheet used to do forest inventory, characterization of the vegetal cover, soil cover and the regeneration on the field campaigns.



INVENTÁRIO FLORESTAL

Área ____ Data ____ Técnicos ____

Coordenadas ____ Colheita de pinhas (Y/N) ____

Fotos: Copas ☐ Parcela ☐ Detalhe ☐ Outras ☐

Observações

Compasso: Regular ☐ Irregular ☐

Origem: Plantada ☐ Regeneração ☐ Outra ☐

Silvicultura (Observações)

Solo
Mobilização do solo ____
Vale/ Cômoro ____
Topografia ____
Outras ____

Povoamento
Controlo Vegetação ____
Desbaste ____
Corte ____
Outras ____

Avaliação dendrométrica

Nº árvores na parcela ____ Pinhas (Y/N) ____ Abertas (Y/N) ____
Fechadas (Y/N) ____

Nº Parcela	Nº Árvore	Idade (anos)	Altura (m)	Perímetro (cm)	Altura Base Copa (m)	Pinhas	Observações

Cover (%)

Bare soil ____
Litter ____
Ervas ____
Arbustos ____

Regeneração pinheiro ____
Invasoras ____
Árvores ____
Outras ____

Observações ____

Regeneração

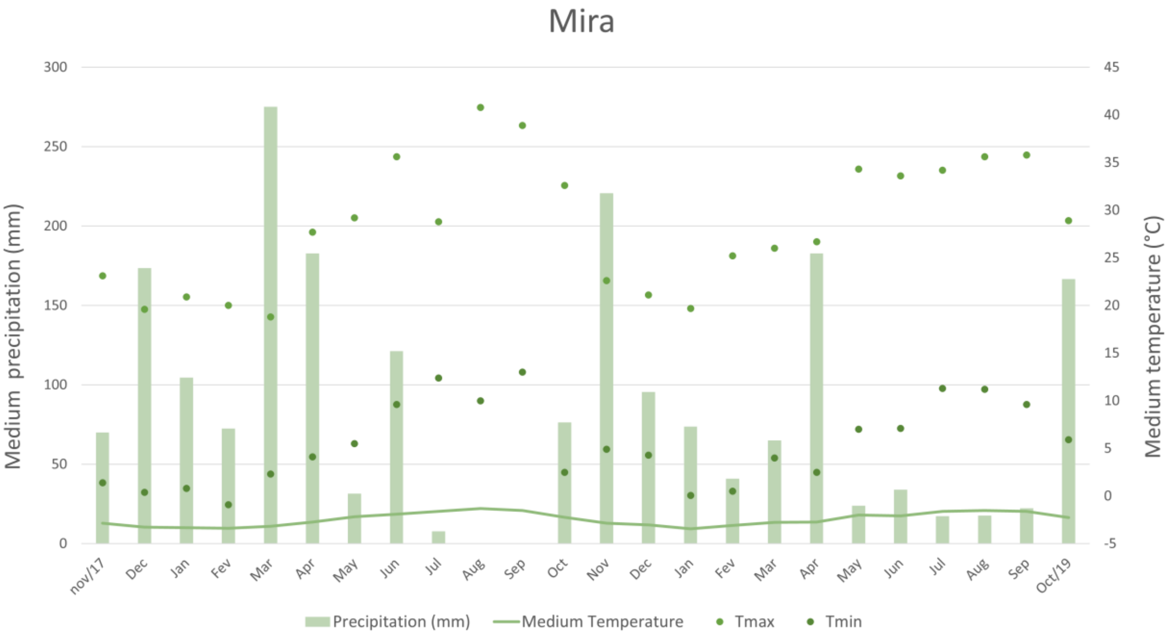
Quadricula	Idade(s)	Densidade	Altura média (cm)	Observações
1				
2				
3				
4				

CROQUI (verso da folha) ☐

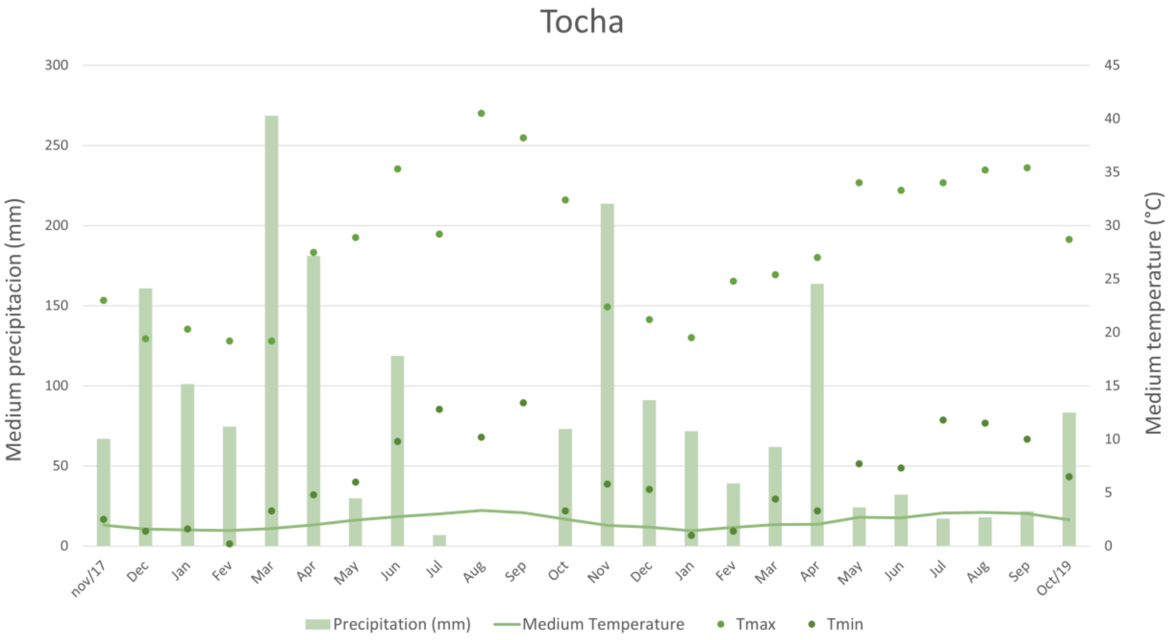
Notas ____

Supplementary Material 2- Medium precipitation (mm), medium temperature (°C), maximum and minimum temperatures (°C), distribution since November 2017 to October 2019, in the selected populations Mira (a), Tocha (b) and Leiria (c).

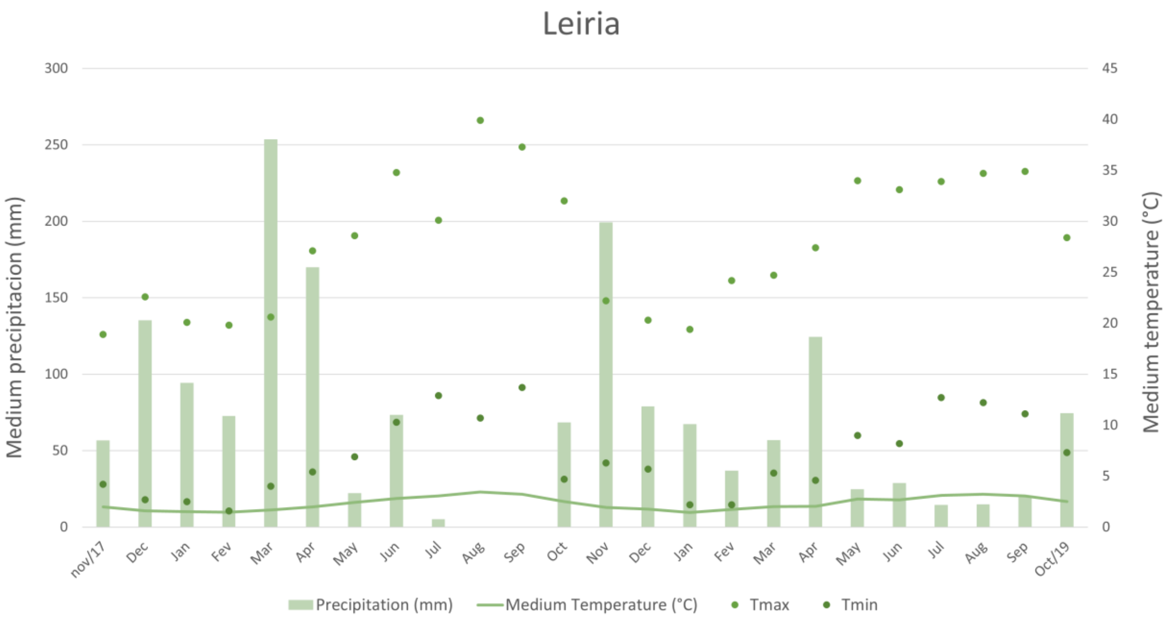
a)



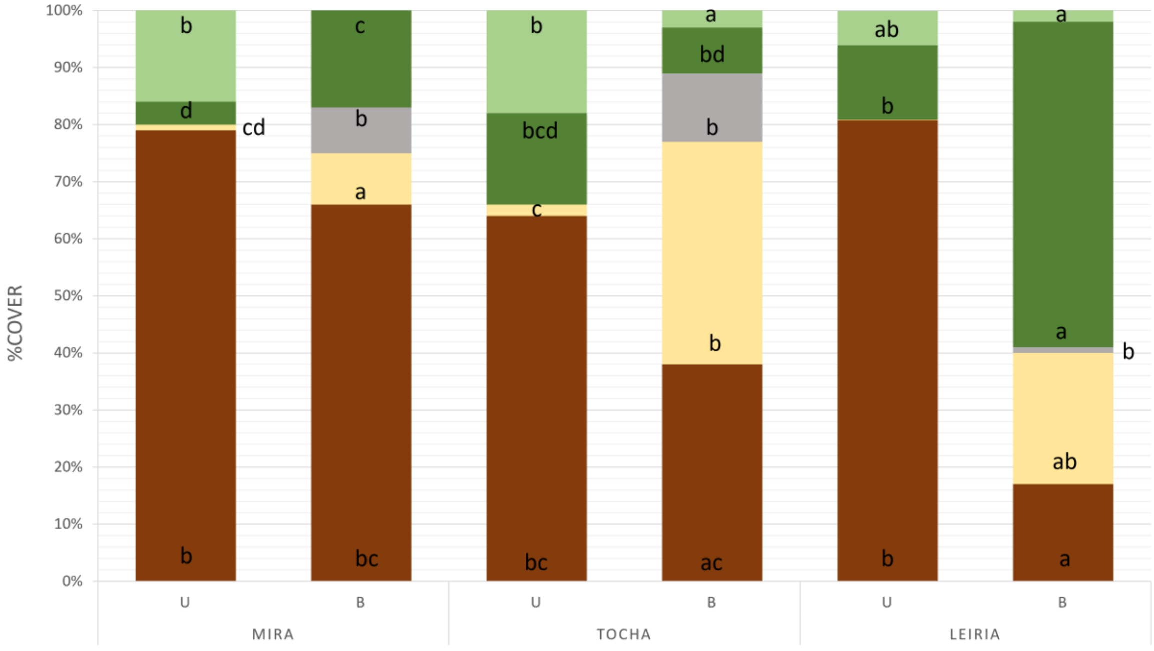
b)



c)

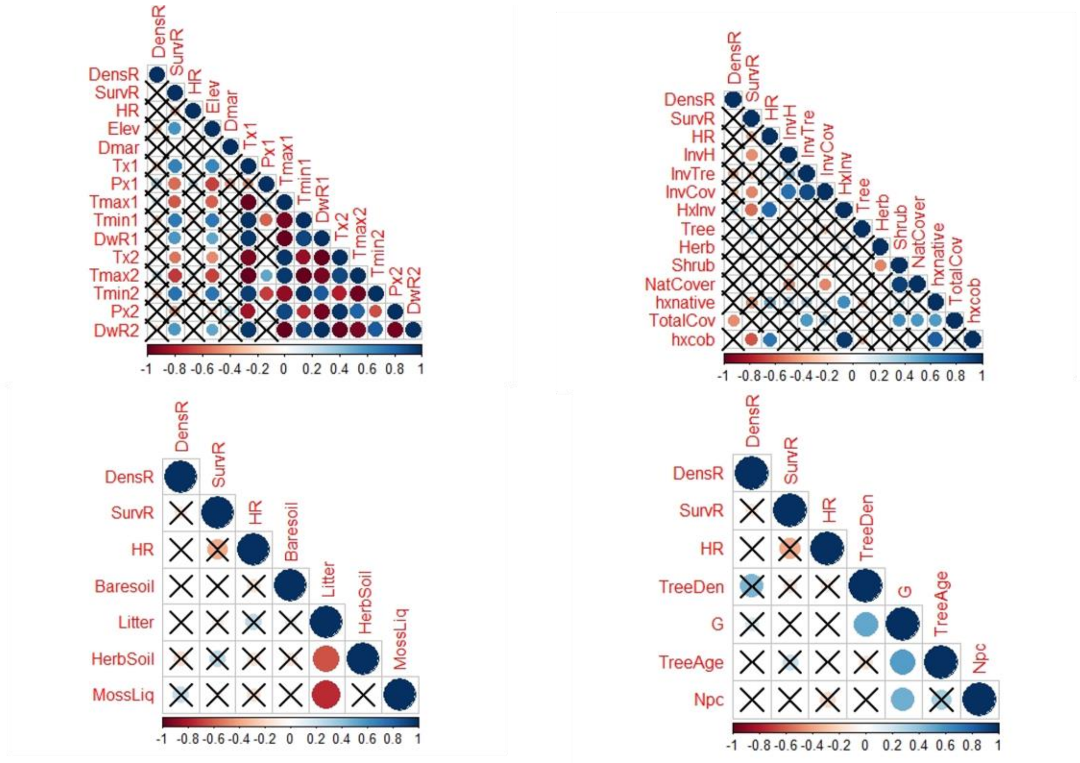


Supplementary Material 3- Soil cover composition by typology on the three locations- Mira, Tocha and Leiria – in unburnt (U) and burnt (B) treatments. Bars represent the mean. Litter (**brown**), Bare Soil (Yellow), Ash (grey), Herbaceous (**dark green**), Mosses and lichens (Light green). The different letter indicates significant differences (p<0.05).



Supplementary material 4 – Graphical representation of all Spearman's correlations tested for the a) unburnt, b) burnt treatments. Negative correlations are shown in red, positive correlations in blue. The X represent p-values>0.05.

a)



b)