Piñas-Bonilla *et al. Annals of Forest Science* *(2024) 81:37*
https://doi.org/10.1186/s13595-024-01258-3

Annals of Forest Science

RESEARCH PAPER **Open Access**

Effects of different loads of *Pinus pinaster* Ait. litter and ash on the germination and growth of *Cistus ladanifer* L

Paula Piñas-Bonilla[1*], Gonzalo Zavala[1], Beatriz Pérez[1] and Belén Luna[1*]

Abstract

Key message *Cistus ladanifer* L. shows an extraordinary plasticity of germination and growth. Fire promotes the regeneration of *C. ladanifer* by triggering its germination through thermal shock as well as by enhancing its seedling growth through the addition of ashes. On the contrary, stacking of *Pinus pinaster* Ait. leaf litter may hinder seedling establishment, at least in the first months after germination.

Context *Pinus pinaster* Ait. is affected by forest fires in the Mediterranean basin. Its extreme flammability is due to the high spatial continuity together with the massive accumulation of leaf litter. *Cistus ladanifer* L. is a species which is widely distributed in the west Mediterranean region where it can form vast shrublands and flourish under these pinewoods. Although high temperatures associated with the occurrence of fire trigger germination of *C. ladanifer*, knowledge on how other factors, such as the presence of litter on the forest floor or the ash left after the fire, influence germination and seedling growth is essential to improve land management plans.

Aims The main objective of this study was to determine the effects of different loads of litter from *P. pinaster* and ash from their combustion on the germination and growth of seedlings of *C. ladanifer*.

Methods Two experiments were carried out to assess the effect of heat shock (100 °C for 10 min) and two loads of litter and ash from *P. pinaster* (control, high and low loads of litter, high and low loads of ash) on the germination and growth after 2 and 4 months of *C. ladanifer*.

Results Heat shock significantly increased the germination of *C. ladanifer*. In contrast, the addition of litter and ash had no effect on total germination but affected the growth of the seedlings coming from seeds exposed to heat shock. Litter treatments reduced biomass of 2 months seedlings and ash increased biomass of 4 months seedlings.

Conclusion *C. ladanifer* is a species favoured by fire in different ways depending on the stage of regeneration. Germination is promoted by heat shock while seedling growth is favoured by ash nutrients. In contrast, pine leaf litter hinders seedling growth, although this effect disappears 4 months after germination. Alternative forest management practices to prescribed fires are recommended if preventing the spread of *Cistus* is a priority.

Keywords Biomass, Cistaceae, Fire, Maritime pine, Seed dormancy, Prescribed fires, Seedlings

---

Handling editor: Paulo Fernandes

*Correspondence:
Paula Piñas-Bonilla
Paula.Pinas@alu.uclm.es
Belén Luna
Belen.Luna@uclm.es
Full list of author information is available at the end of the article

1 Introduction

Pine forests occupy vast areas of the Mediterranean basin, especially in locations poor in nutrients and/or water (Keeley and Zedler 1998). Moreover, the distribution of these species throughout the Mediterranean has been favoured by reforestation in the second half of the twentieth century, contributing to an increase in the risk of fires (Pausas et al. 2008; Wittenberg and Malkinson 2009). Prominent among the different species is the maritime pine (*Pinus pinaster* Ait.) which is widely distributed in the western Mediterranean and was the second most affected species by forest fires (FF) in the Iberian Peninsula between 2006 and 2015 (López-Santalla and López-García 2019). *P. pinaster* forests are very flammable due to their structure and continuity and the accumulation of leaf litter (Elvira and Hernando 1989; Fernández-Guisuraga et al. 2021), which means that fires can potentially be extremely severe in dense forests (Fernandes and Rigolot 2007; Gómez-Vázquez et al. 2013). Furthermore, there are often tall and dense shrubs in the undergrowth of these pine forests (Taboada et al. 2018), which increases the vertical continuity of the fuel, favouring fire in the treetops and enhancing its intensity (Fernandes and Rigolot 2007).

However, although fire is a natural ecological factor that contributes to the dynamics of the Mediterranean ecosystems, in the last few years the fire risk has increased due to enhanced temperatures and severe drought associated to climate change (Moriondo et al. 2006; Jones e al. 2022). To date, the main policy for fighting FF in the Mediterranean basin has been to extinguish them, which, although it achieves the main objective, leads to a gradual increase in the interannual loads and continuity of the fuel, which in turn leads to a landscape which is even more susceptible to fires (Pausas and Fernández-Muñoz 2012). The need has therefore been seen of developing preventive tools in the fight against forest fires that decrease the fuel and its continuity. Some of these management practices include clearing and prescribed fires, of proven efficiency for reducing the severity of the FF and improving the resilience of areas treated to prevent them (Fernandes and Botelho 2003; Waltz et al. 2014; Duane et al. 2019). However, the effects of prescribed fires on the regeneration of the shrubs that grow in these pine woodlands are not clear. Some studies show that in the pine stands with a high density of trees, the undergrowth decreases and disappears for lack of light (Facelli and Kerrigan 1996), for the allelopathic properties of the pines (Valera-Burgos et al. 2012) or because of the changes in the physical–chemical characteristics of the soils (Fernández-Marcos 2022). In contrast, in pine stands with a lower density, the shrubs flourish and species of Ericaceae, Leguminosae and Cistaceae among others, coexist (Vasques et al. 2012; Taboada et al. 2018). Specifically, Cistaceae can form vast areas of shrubland, especially after a fire (Trabaud and Renard 1999). This family displays seeds with a hard seed coat (Thanos et al. 1992) which prevents germination until a scarifying agent, like the high temperatures reached during a fire, permit their rupture and, thus, the entry of water to the embryo and its subsequent germination (Baskin and Baskin 1998).

This study will deepen knowledge of the regeneration of *C. ladanifer*, which is one of the most widespread species in the Iberian Peninsula and is considered a pyrophyte (Naveh 1974) or generalist and opportunistic coloniser (Trabaud 1995). It occupies strongly disturbed areas associated with early successional stages and is capable of colonising highly disturbed soils, like those present in areas affected by fire, abandoned agricultural land or disturbed woods (Herrera 1984; Gallego et al. 2020). *C. ladanifer* forms monospecific shrublands that often do not evolve into more mature stages of vegetation (Mendes et al. 2015) due to their great capacity for regeneration together with the allelopathic effect of their leaves which inhibit the development of other plants (Tárrega et al. 2001; Herranz et al. 2006; Sosa et al. 2010). Similarly, these homogeneous landscapes present a great accumulation and continuity of dead fuel, which leads to situations of high flammability (Saura-Mas et al. 2010; Baeza and Santana 2015).

Detailed knowledge exists on the effect of high temperatures associated with the event of a fire in the increase in germination of *C. ladanifer* (Thanos et al. 1992; Valbuena et al. 1992; Herranz et al. 1999; Luna et al. 2019). However, although many studies have dealt with the effect of heat shock on germination, little is known about other environmental factors that might influence the emergence and growth of this species. For example, the regeneration of *C. ladanifer* is associated with disturbed surroundings (Luis-Calabuig et al. 2000; Tárrega et al. 2001), but knowledge of the specific factors that trigger or inhibit the different stages of establishment, such as germination and seedling growth, can help to better understand the ecological requirements of the species. In general, leaf litter or the remains which lie on the surface of the soil can interact with the germination of seeds via different mechanisms (Tormo et al. 2020). The effects of leaf litter on the establishment of seedlings are complex (Facelli and Pickett 1991; Koorem et al. 2011) and vary considerably among studies depending on the vegetation variable studied, the study method, the duration of the experiment, latitude, habitat, type and amount of litter and target species (Xiong and Nilsson 1999). Leaf litter can facilitate the establishment of some species by improving availability of water (West 1979; Fowler 1986), decreasing competition due to lower plant density (Sydes and Grime 1981; Facelli and Pickett 1991; Myster 1994), buffering rapid fluctuations of temperature or preventing predation by small vertebrates like rodents (Shaw 1968; Grime et al. 1981). In contrast, the establishment of many species is negatively affected by shade (Facelli and Pickett 1991; Roy and Sonie 1992), the physical barrier (Herranz et al. 1999; Izhaki et al. 2000), the reduction in the temperature amplitude on the soil or its physical–chemical changes (Binkley and Giardina 1998; Valera-Burgos et al. 2012; Gallego et al. 2020). Moreover, all these effects depend on the local microenvironment, the physical and chemical characteristics of the leaf litter (Koorem et al. 2011) as well as the size of the seeds and their physiological responses to light and temperature (Vázquez-Yanes et al. 1990; Facelli and Pickett 1991; Peterson and Facelli 1992; Facelli and Kerrigan 1996). While in mesic environments leaf litter limits seedling establishment by reducing light, in dry environments, leaf litter enhances seedling establishment and growth by increasing water availability (Fowler 1986). In any case, we can generally say that the short-term effects of leaf litter on regeneration are usually negative (Xiong and Nilsson 1999), which would imply that the development of the seedlings depends to a great extent on the factors that reduce leaf litter like rapid decomposition and disturbances. In the case of pine forests, the low rates of decomposition imposed by the physical–chemical characteristics of the leaf litter will condition the growth of some species of underwood, and the elimination of the leaf litter in this case will be fundamentally facilitated by fires. The main direct effects of fire include the high temperatures reached and the deposition of ash. As mentioned above, the heat shock associated with the fire can break the physical seed dormancy of some Cistaceae triggering germination and provoking the massive establishment of seedlings after the fire has passed (Arianoutsou and Margaris 1980; Trabaud and Lepart 1980; Moreno et al. 2011). The ash can provide nutrients for the plants but can also increase the pH of the soil (Henig-Sever et al. 1996) and its osmotic potential (Ne'eman et al. 1992), which can inhibit the germination and growth of the seedlings (Ne'eman et al. 2009). The amount of deposited ash can also condition these effects, normally decreasing germination as it increases (Reyes and Casal 2004; for a review see Fernández-Marcos 2022).

Although direct fire effects on *C. ladanifer* germination are quite well known, there is not information about the influence of litter on the forest floor or the ash that is left after the fire on its germination and seedling growth. To our knowledge, this study analyses for the first time the effects of these factors on the germination and growth of *C. ladanifer*. A detailed knowledge of undergrowth species regeneration, such as *C. ladanifer*, is needed to understand the post-fire dynamics of vegetation and fuels in forests. Additionally, this more in-depth knowledge will help to give effective guidelines for landscape management under the current context of increasing fire risk. The main objective of this study was to determine the effects of different loads of leaf litter from *Pinus pinaster* and ash from their combustion on the germination and growth of seedlings of *C. ladanifer.* The aim was also to assess if these effects are similar between seeds that have or have not been exposed to heat shock. The factors analysed in this research are related both to the natural conditions and to different management methods. The different loads of fuel can be related to pine stands of different densities, but also to different clearance treatments. Similarly, both the exposition to heat shock and the addition of ash could be related to a fire or the application of prescribed fires, and thus, the results obtained would have implications for the ecological interpretation of the regeneration of *C. ladanifer* and the management of forests in which they are the main species.

Since seeds of *C. ladanifer* have physical dormancy and establish abundantly after fire, we hypothesised that germination increases after exposure of seeds to heat shock and seedling growth is favoured by ash addition. On the contrary, a negative effect of litter on seed germination and seedling growth would be expected because of the physical barrier exerted on the tiny seeds of *C. ladanifer*. To test these hypotheses, we set the following specific objectives: (1) to determine the effects of high and low litter and ash loads on the germination of *C. ladanifer*, (2) to test whether a heat shock modifies the germination response to the above treatments, (3) to establish the effects of the same treatments, high and low litter and ash loads, on the growth of *C. ladanifer* seedlings, (4) to identify whether growth patterns are similar between seedlings from previously heated vs. unheated seeds, and (5) to explore the effects of all these treatments on the 2- and 4-month-old seedlings growth.

2 Material and methods

The seeds used in this study were gathered in the municipality of Navalucillos, located in the centre of the Iberian Peninsula (Coordinates UTM 30N: X:348.273; Y: 4.385.357). This zone is characterised by a Mediterranean climate with very cold winters and hot and dry summers, and a mean annual temperature of 16.9 °C and mean annual rainfall of 309 mm (AEMET 2023). The zone is occupied by a *Pinus pinaster* Ait. stand which displays zones with different densities of trees, ranging from 1211 trees/ha to 217 trees/ha, also including different densities of *C. ladanifer* (0.03% and 25.84% of ground cover in high- and low-density zones, respectively). Leaf litter loads were determined in four plots of 30 × 30 m in both high- and low-density pine stands. In each plot, four 50 × 50 cm quadrat samples were set, and litter was collected, dried and weighted in the laboratory. Leaf litter loads were 1022 ± 53 and 457 ± 66 g/m^2 respectively for high- and low-density pine stands.

In July 2021, during the period of seed dispersal, capsules were gathered from 50 *C. ladanifer* shrubs. They were placed in paper envelopes and taken to the laboratory where the seeds were extracted, cleaned and stored in paper bags. The seeds were kept in the dark in laboratory conditions at ambient temperature. Leaf litter from *P. pinaster* was also gathered from the ground, cleaned, dried and stored until the beginning of the experiment.

2.1 Experimental design

Seeds were exposed to a heat shock of 100 °C for 10 min in an air-forced oven since Luna et al. (2023) found that this temperature and exposure time led to high germination rates. Half the seeds were exposed to heat shock (H+) and the other half were not (H−). After the heat shock, both groups of seeds were left to germinate under different treatments simulating the conditions in the high- and low-density pine stands from which the leaf litter load had been estimated in 1000 g/m^2 for high density and 450 g/m^2 for low density pine stands.

The treatments consisted in the addition of leaf litter simulating the loads found in the field: a high load of needles (↑Lit) and low load of needles (↓Lit) corresponding to high- and low-density pine stands, respectively. Equally, two treatments with ash were applied which corresponded to the quantity of ash generated by a fire in the high- and low-density pine stands studied. To this end, the loads corresponding to the high and low amount of litter were burned simultaneously in a fire research laboratory under similar conditions. We obtained 36 g/m^2 ashes in the case of a high load (↑Ash) and 18 g/m^2 ashes for a low load (↓Ash). The last treatment consisted in a control without the addition of litter or ash (Con) (Appendix Fig. 7).

2.2 Experiment 1: Germination

To study the germination of *C. ladanifer*, 30 aluminium trays measuring 30 × 30 cm were sown with the seeds that had previously received the heat shock (H+), and 30 similar trays were planted with the seeds which had not (H−). Each tray was planted with 25 seeds of *C. ladanifer* with vermiculite as the substrate and the treatments were applied on the surface of the vermiculite making a total of 6 replicas for each of the treatments (Con, ↓Lit, ↑Lit, ↓Ash, ↑Ash). The trays were kept for 8 weeks in a greenhouse. During this time, the maximum temperatures did not exceed 27 °C and the minimum temperatures did not drop below 11 °C. The seeds were watered daily and the number of germinated seeds were counted three times per week and removed from the tray. A seed was considered to have germinated when the seedling had emerged above the substrate. All the trays were moved weekly to avoid the possible effects of location.

Germination was characterised by two variables: the total percentage of germination (total germination) and the time elapsed from planting to germination, that is the number of days until the first seed germinated (T_0). The effects of heat shock (H− and H+) and the treatments (Con, ↑Lit, ↓Lit, ↑Ash and ↓Ash) on the total germination of *C. ladanifer* were analysed using a generalised linear model (GLM). Based on the structure of the data, we used a binomial distribution and a logit link for the total germination. In the case of T_0, the residues of the analysis did not fulfil the criteria of normality and homoscedasticity either for the gross variables or the transformed variables, so the effect of the treatments was calculated separately for the seeds that had received the heat shock and those that had not, using the Kruskal–Wallis non-parametric test. After analysing the total germination and T_0, a pairwise comparison was performed with a Bonferroni post hoc test.

2.3 Experiment 2: Growth

Growth of *C. ladanifer* was analysed after 2 and 4 months from the start of the experiment. The seeds were planted in seedbeds of 30 cells (5.5 × 5.5 × 16 cm) with vermiculite as the substrate. Each seedbed contained 6 replicas of each of the 5 treatments, which were randomly placed in the seedbed before starting the experiment. Eight seedbeds were used in the case of the heat shock seeds (H+). The seeds that had not been exposed to the heat shock (H−) had more difficulty to germinate so that 10 seedbeds were used instead of 8. If all the seeds emerged, total number of replicas would be 48 seedlings for each of the 5 treatments with heated seeds subjected to heat shock (H+) and 60 seedlings for each of the treatments with unheated seeds (H−). Moreover, in an attempt to ensure the emergence of at least one seedling per cell, two seeds were planted in each one. When both seeds germinated the last plant to emerge was removed from the cell. During the experiment, the seedlings were kept in the greenhouse under the same conditions as in experiment 1. They were watered daily and once a week all the seedbeds were moved to ensure that they all were exposed to the same conditions of light and water. Two months after the start of the experiment four H+ seedbeds were randomly selected. The seedlings were extracted, cleaned and measured for total, aerial and root length. After measurement, they were dried in the oven at 75 °C for 48 h. The dried seedlings were weighed differentiating total, aerial, leaf, stem and root biomass. After

4 months, the procedure was repeated with the 4 remaining H + seedbeds. The number of seedlings emerged from seeds that had not received the heat shock was very low (Con:11,↑Lit:4,↓Lit:4,↑Ash:8 and ↓Ash: 14 seedlings) and thus, all of these seedlings were analysed after 2 months.

Growth of the seedlings was described with the variables, total, shoot and root length, and total, aerial, leaf, stem and root biomass of each seedling. Given the low number of seedlings that had emerged from seeds that had not been exposed to heat shock, it was not possible to analyse the effect of heat shock on growth. Thus, the effects of the treatments were analysed separately for plants exposed to heat shock and those that were not. The effect of the treatments on the growth of the seedlings was analysed with one-way ANOVA with 5 levels (Con, ↑Lit, ↓Lit, ↑Ash and ↓Ash). In the case of the variables that did not fulfil the criterion of a normal distribution of the data, a Kruskal–Wallis nonparametric test was performed. When significant differences ($p \leq 0.05$) were found between treatments, Bonferroni's post hoc pairwise comparison was used.

All the statistical analyses were conducted with SPSS Statistics version 24.0 (SPSS, Chicago, IL, USA). The data on which the analyses are based are available in a repository (Piñas-Bonilla et al. 2024).

3 Results

3.1 Experiment 1: Germination

Heat shock played a fundamental role in the germination of *C. ladanifer*, as the seeds that were exposed to heat shock reached almost 100% germination, while non exposed seeds reached barely 5% germination (Fig. 1). In contrast, the other treatments had no effects on total germination. Moreover, no interaction was detected (Fig. 1, Table 1).

The start of the germination (T_0) of the seeds exposed to heat shock was significantly affected by the treatments ($p < 0.001$). Germination began later in the treatments with litter than with the control and the treatments with ash (Fig. 1, Appendix Fig. 8). In the case of seeds not exposed to heat shock, T_0 was not affected by the different treatments (Table 2).

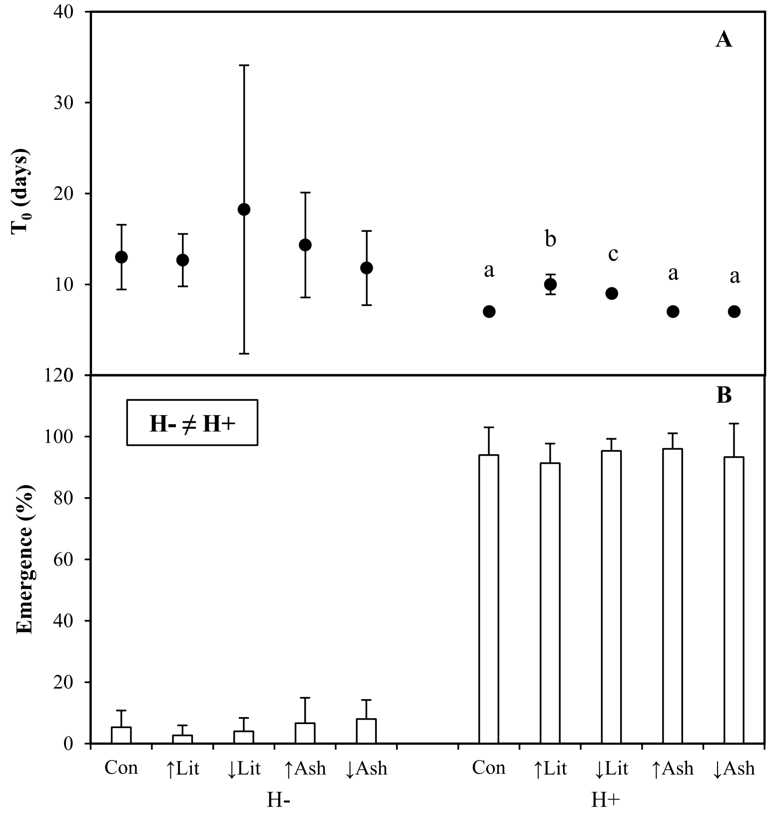

Fig. 1 Percentage of total germination (white columns) and T_0 or number of days that passed until the beginning of germination (black dots) (mean + sd) for H – (seeds not exposed to heat shock) and H + (seeds exposed to heat shock). The seeds previously exposed or not to heat shock were left to germinate under five different treatments Con: control; ↑Lit: high load of litter; ↓Lit: low load of litter; ↑Ash: high load of ash; ↓Ash: low load of ash. The letters indicate significant differences ($p \leq 0.05$) according to Bonferroni's post hoc pairwise comparison. H – ≠ H + indicates that total germination was significantly lower in the seeds not exposed to heat shock compared to those that were

Table 1 Results of the general linear model (GLM) analysis for the main effects of the heat shock, the treatments and their interactions on the total germination of the *Cistus ladanifer* seeds

Total germination		
	χ^2	p
Heat shock	2684.00	<0.001
Treatment	2.99	0.056
Heat shock x Treatment	1.23	0.087

Table 2 Analysis of the effects of the different treatments on the T_0 (number of days required to start germination) of the *Cistus ladanifer* seeds exposed (H+) and not exposed (H−) to heat shock. The data did not follow a normal distribution so the non-parametric Kruskal–Wallis test was conducted

		T_0	
		H	p
Treatment	H+	28.079	< 0.001
	H−	0.758	0.944

3.2 Experiment 2: Growth

The growth of the seedlings from seeds that received heat shock revealed significant differences among the different treatments both after 2 and 4 months from the start of the experiment (Table 3). After 2 months, the total length of seedlings grown with the high litter load (↑Lit) was similar to those grown with the low litter load (↓Lit), but shorter than those grown with the other treatments (Con, ↑Ash and ↓Ash) (Table 3, Appendix_Table5). This difference was mainly due to the root length, which similar to the total length, was not significantly different from the root length of the seedlings under a low load of litter but was significantly shorter than the rest (Fig. 2). After 4 months, while the total, aerial and root length of the seedlings that grew under the treatment of the litter equaled that of the control (Fig. 2), the total length of the seedlings from the treatment of a low load of ash was significantly longer than the control and those from the treatment with litter (Appendix_Table 5).

After 2 months, the seedlings from the two leaf litter treatments were significantly lighter than the rest of the seedlings, both in aerial and root biomass (Fig. 3). Leaf biomass investment was also significantly lower in these two treatments compared with the others (Fig. 4). In addition, stem biomass was significantly lower in the treatment with high litter load (Fig. 4, Appendix Table 5). After 4 months, seedling biomass of the litter treatments was equal to that of the control. However, the biomass (total, aerial, root, leaf and stem) of the ash-treated seedlings was significantly greater (Figs. 3 and 4, Table 3).

The investment between roots and shoots was different for length and biomass variables. Root length (7.22 ± 0.50 cm) was around the triple than shoot length (2.44 ± 0.96 cm). On the contrary, root biomass (0.70 ± 0.14 mg) was one third of the shoot biomass (2.35 ± 0.51 mg) (Figs. 2 and 3).

Seedlings survival analyses was not among the objectives of our study, but we want to point out that it was very high, around 100% for all treatments except for seedlings grown under the high load of litter (↑Lit), in which seedling survival decreased to 85%.

In the case of the seedlings from non-heat shocked seeds, no statistically significant differences in growth were found among treatments (Table 4). However, in some cases there were marginally significant differences ($p<0.1$), showing similar growth patterns to those of seedlings from heat shocked seeds. Seedlings grown at high litter load showed less root length development compared to the other treatments (Fig. 5, Appendix Table 6). With respect to the biomass, seedlings treated with ash, especially those

Table 3 Analysis of the effects of the treatments on the different variables of the growth of the *Cistus ladanifer* seedlings from seeds exposed to heat shock, after 2 and 4 months from the start of the experiment. If the data followed a normal distribution, a parametric one-way ANOVA was performed (Δ). However, if the data did not follow a normal distribution the non-parametric Kruskal–Wallis test was used (□)

	2 months			**4 months**		
	Statistic	p		**Statistic**	p	
Total length	4.607	0.002	Δ	7.441	< 0.001	Δ
Aerial length	3.201	0.016	Δ	3.066	0.020	Δ
Root length	4.743	0.002	Δ	16.842	0.002	□
Total biomass	55.726	< 0.001	□	41.120	< 0.001	□
Aerial biomass	51.030	< 0.001	□	39.419	< 0.001	□
Stem biomass	42.859	< 0.001	□	46.394	< 0.001	□
Leaf biomass	52.624	0.021	□	36.766	< 0.001	□
Root biomass	18.550	< 0.001	Δ	41.044	< 0.001	□

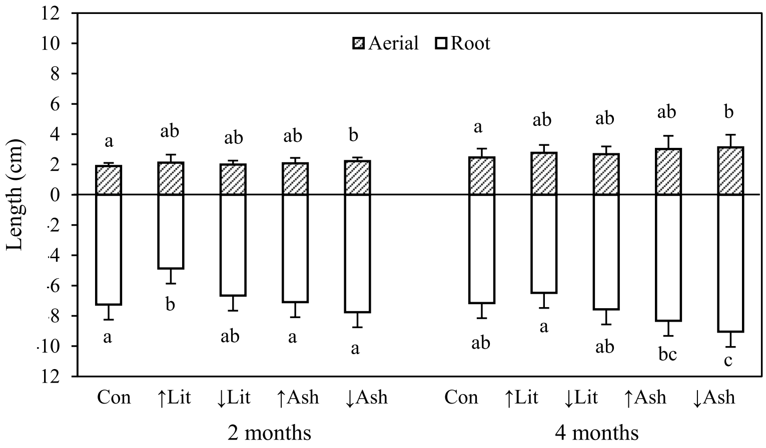

Fig. 2 Aerial and root length of the *Cistus ladanifer* seedlings (mean + sd) after 2 and 4 months of growth under greenhouse conditions. The seeds of these seedlings were exposed to heat shock before the start of the experiment and were subsequently left to germinate under five different treatments: Con: control; ↑Lit: high load of litter; ↓Lit: low load of litter; ↑Ash: high load of ash; ↓Ash: low load of ash. The different letters show significant differences (p ≤ 0.05) among the different treatments after 2 and 4 months based on Bonferroni's pairwise comparison

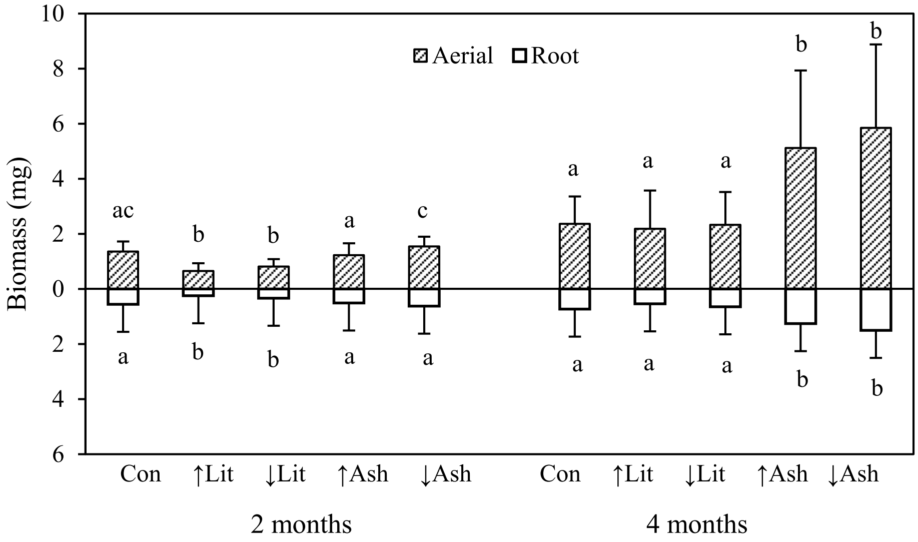

Fig. 3 Aerial and root biomass of the *Cistus ladanifer* seedlings (mean + sd) measured after 2 and 4 months of growth in greenhouse conditions. The seeds of these seedlings were exposed to heat shock before the start of the experiment and were subsequently left to germinate under five different treatments: Con: control; ↑Lit: high load of litter; ↓Lit low load of litter; ↑Ash: high load of ash; ↓Ash: low load of ash. The different letters show significant differences ($p \leq 0.05$) among the different treatments after 2 and 4 months based on Bonferroni's pairwise comparison

subjected to a low ash load, showed a greater development of the aerial part (Fig. 6, Appendix Table 6).

4 Discussion

The results of this study show that heat shock is fundamental in the germination of *C. ladanifer*, independently of the subsequent treatment applied. This finding coincides with other studies where leaf litter or ash had no effects on the percentage of total germination of other species of *Cistus* or *Pinus* (Escudero et al. 1997; Trabaud and Renard 1999; Herrero et al. 2007). However, a negative effect of leaf litter and ash is frequently found on the germination of different taxa including *Cistus* (González-Rabanal and Casal 1995; Facelli and Kerrigan 1996; Henig-Sever et al. 1996; Izhaki et al. 2000; Reyes and Casal 2004). This negative effect, both of leaf litter and ash, depends on the layer thickness (Koorem et al. 2011; Bodí et al. 2014), and in most cases, there is a greater inhibition of the germination of the seedlings with greater loads of litter and ash (Trabaud and Casal 1989; Ne'eman et al. 1992; Reyes and Casal 1998; Tormo

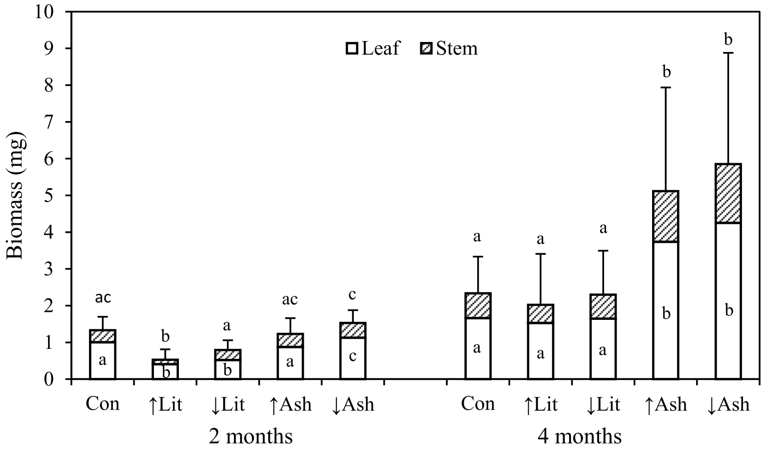

Fig. 4 Leaf and stem biomass of *Cistus ladanifer* seedlings (media + sd) measured after 2 and 4 months of growth under greenhouse conditions. The seeds of these seedlings were exposed to heat shock before the start of the experiment and were subsequently left to germinate under five different treatments: Con: control; ↑Lit: high load of litter; ↓Lit: low load of litter; ↑Ash: high load of ash; ↓Ash: low load of ash. The different letters show significant differences ($p \leq 0.05$) among the different treatments after 2 and 4 months based on Bonferroni's pairwise comparison

Table 4 Results of the non-parametric Kruskal–Wallis test for the main effects of the treatments of the seedlings from seeds not exposed to heat shock on the different variables of the *Cistus ladanifer* seedlings after 2 months

	2 months	
	H	***p***
Total length	2.781	0.595
Aerial length	2.621	0.623
Root length	8.883	0.064
Total biomass	8.739	0.068
Aerial biomass	8.166	0.086
Stem biomass	4.522	0.34
Leaf biomass	8.435	0.077
Root biomass	8.911	0.063

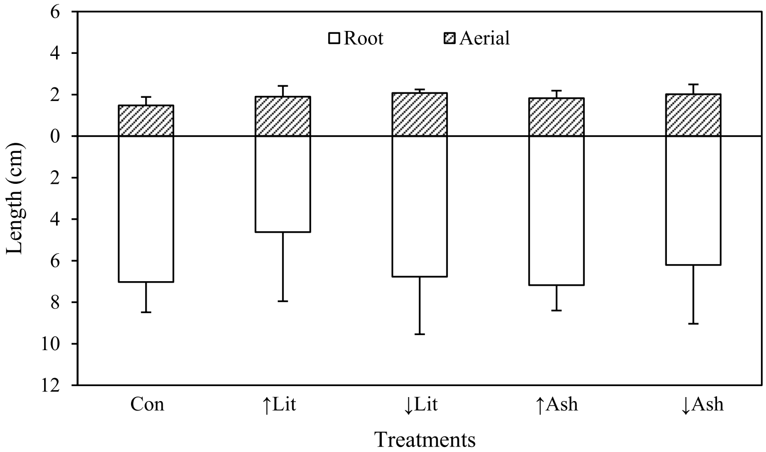

Fig. 5 Aerial and root length of the *Cistus ladanifer* seedlings (mean + sd) from seeds that were not exposed to heat shock. These measures were taken after 2 months of growth under greenhouse conditions. The seed of these seedlings were directly left to germinate under five different treatments: Con: control; ↑Lit: high load of litter; ↓Lit: low load of litter; ↑Ash: high load of ash; ↓Ash: low load of ash

2020). As well as the load, other factors can determine the effect of leaf litter on germination, like the type of leaf litter (Koorem et al. 2011), or the size and shape of the seeds (Facelli and Pickett 1991). However, in our study, none of the loads of litter or ash from *P. pinaster* had an effect on the total germination of *C. ladanifer*, which reflects the great germinative capacity of this species in spite of the small size of its seeds (0.27 mg) (Luna et al. 2022). The start of the germination also did not vary among treatments for those seeds not exposed to heat shock. Since the total germination for unheated seeds was very low, the analysis of T_0 was based on a very low number of germinated seeds, which probably led to a high intra-group variance and consequently to lack significant differences. Although in our study total germination was not affected, the start of

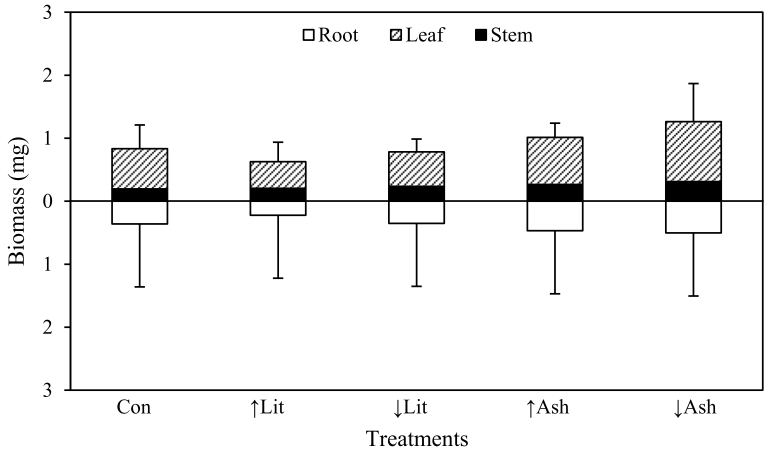

Fig. 6 Leaf, stem and root biomass of the *Cistus ladanifer* seedlings (mean + sd) from seeds that were not exposed to heat shock. These measures were taken after 2 months of growth under greenhouse conditions. The seed of these seedlings were directly left to germinate under five different treatments: Con: control; ↑Lit: high load of litter; ↓Lit: low load of litter; ↑Ash: high load of ash; ↓Ash: low load of ash

germination did differ among treatments for those seeds exposed to heat shock, with the seedlings that grew with the leaf litter treatments being the slowest to begin to emerge. This could be due to the physical barrier that they have to overcome to get to the light (Koorem et al. 2011). Moreover, this physical barrier could provoke changes in the growth of the seedlings after they emerge (Reyes and Casal 2004). In this respect, we found that although the addition of the litter and ash did not affect the final germination of *C. ladanifer*, it did affect the growth of the seedlings.

The effect of heat shock and its interaction with the other treatments on the growth of the seedlings could not be statistically assessed due to the low germination rate of seeds not exposed to heat shock. Although new research is needed to verify this issue, our data give us a glimpse that growth patterns, related to length and biomass, appeared quite similar between seedlings from seeds exposed and not exposed to heat shock. A similar behaviour has been observed in other species from the Mediterranean basin, specifically of the *Cistus* genus, like *C. creticus* and *C. salviifolius*, with no effects from heat shock on the growth of the seedlings (Hanley and Fenner 1998). Equally, the distribution between the aerial length and the root length was similar independently of the treatment received, and different between the variables of length and biomass. While the root length was greater than the aerial length, the biomass was greater in the aerial biomass than the root biomass. This means that the specific root length (SRL) developed by unit of biomass was high, which gives the seedlings greater efficacy in the acquisition of resources (water, nitrogen and phosphorus) (Reich et al. 1998; Comas et al. 2002; Padilla and Pugnaire 2007) and a higher rate of survival when faced with the summer drought (Paula and Pausas 2011). Furthermore, the greater investment of resources in the aerial biomass would imply higher rates of growth, and thus, quicker establishment (Verdú 2000). In short, these characteristics are advantageous in Mediterranean environments, where rapid growth and a good development of roots are a key to successfully facing the summer drought.

The growth of *C. ladanifer* is depending on the age of the seedlings. After 2 months, it was found that the total and root length of the seedlings that grew with a high load of litter were shorter than those of the rest of the treatments. Similarly, the total, aerial and root biomass of the seedlings grown with both treatments of litter was smaller than those from other treatments. These results coincide with those found for other species (Hamrick and Lee 1987). A thick layer of litter will cause the seedlings reach the light later and thus the onset of photosynthesis may be delayed, which would reduce the photosynthates to be sent to the roots. This smaller root growth can have serious consequences for its survival and establishment in zones lacking in water and nutrients, as in the case of Mediterranean environments (Green et al. 2006; Herranz et al. 2006; Gallego et al. 2020), which could even cause them to die. This coincides with the results of our study where greater mortality was observed in the seedlings that grew under the treatment with a high load of litter. In woods with a high load of litter, the rates of decomposition are low, which mean that they stay on the ground for a long time (García-Plé et al. 1995). The physical barrier presented by the layer of litter can serve as protection against direct impact of rain or changes in temperature (Gallego et al. 2020), but negative effects have mostly been described as they hinder the establishment of the seedlings and make their growth difficult (Facelli and

Kerrigan 1996; Koorem et al. 2011). It was also observed that although a low load of leaf litter (< 100-300 g/m^2) can lead to an increase in production and diversity, a high load (> 900 g/m^2) produces negative effects on diversity (Carson and Peterson 1990). In our study, there were no statistically significant differences in growth (length and biomass) between the seedlings treated with the two loads of litter (450 and 1000 g/m^2), but a pattern was observed of the consistent gradual effect of the load which worsened growth the greater the load.

While the seedlings that were with the litter showed less growth after 2 months, by 4 months they had equaled those of the control. This was probably related to the longer time that they needed to emerge from the layer of litter, but once they managed to emerge and reach the light, the negative effects of the layer of litter disappeared. This could lead us to think that the negative effects of the litter were due to the physical obstruction that they presented, and not so much to their possible allelopathic effect, because in that case it would have lasted over time. These negative effects together with the long permanence of the leaf litter without decomposing could explain, at least in part, that the zones with a high density of pines have a lower density of shrubs in the undergrowth as can be inferred from our results and observed in the study area with the different tree densities.

These high-density areas are extremely flammable, due to the great accumulation of fuel on the ground, which carries a high risk of fires; and in the case that they do occur, of high-intensity fires (Fernandes and Rigolot 2007). Thus, they are zones in which preventive measures, like the use of prescribed fires, should strategically be implemented to reduce the risk of fire and its severity (Fernandes and Botelho 2003). After a fire, the dead fuels turn into large quantities of ash that are deposited on the ground. In general, the ash could enhance the growth of the seedlings as they contribute nutrients, especially nitrogen (Downes et al. 2013). However, they could also have negative effects on the recovery of the species present in the undergrowth (Reyes and Casal 1998), either due to the increase in pH (Henig-Sever et al. 1996; Izhaki et al. 2000) or the osmotic potential (Ne'eman et al. 1992; González-Rabanal and Casal 1995). In our study, the ash in general had a positive effect on the growth of *C. ladanifer*. Although there were scarcely statistically significant differences between the two loads of ash applied, this positive effect was more evident with a low load of ash, and especially after 4 months. This could be related to the greater nutritional needs of the larger seedlings and with the nutritional contribution implied by the ash. In general, most of the literature focuses on the effects of ash on the germination and not on the growth of the seedlings, which makes it difficult to compare our findings with the existing bibliography (Fernández-Marcos 2022) and highlights the need for more research on this question. As well as being scarce, these studies report different results, finding that the addition of ash either had no effect on the growth (length and biomass) of several species of *Pinus* (Reyes and Casal 2004), or produced a decrease in the aerial and root growth in the treatments with a deeper layer or load of ash in all the species studied like *C. salviifolius*, *C. creticus* and *Pinus halepensis* (Ne'eman et al. 1993). Moreover, these harmful effects were greater in the cistaceae than in the pines (Ne'eman 1997). In this research, benefits from the ash were found in the growth of the seedlings in the first months, which is fundamental for their establishment as it is the most critical stage that conditions the establishment of the plants and the dynamic of vegetable populations (Facelli 1994).

The study of the effect of different treatments on the germination and growth of *C. ladanifer* is fundamental to be able to understand the dynamic of the undergrowth in *P. pinaster* forests and plantations. Many species of *Cistus* store their seeds on the ground, and after the fires, they experience massive germination as a recolonising strategy (Frazão et al. 2018). This study evidences the extraordinary plasticity for germination and growth of *C. ladanifer* which seems to show a great capacity for adapting to different environments (Núñez-Olivera et al. 1996). Our findings improve the knowledge on the regeneration of this species and make it possible to prudently infer some implications for making decisions on the most effective treatments when managing *P. pinaster* pine stands and the vegetation underneath when facing adverse conditions like forest fires. Well-developed undergrowth could mean an increase in diversity but could also lead to an increase in the horizontal and vertical continuity of the fuel, which would favour the severity and intensity of the forest fires (Baeza and Santana 2015). Thus, it is essential to seek a balance between maintaining diversity and reducing the severity of forest fires. Different preventive management techniques are used to reduce the negative impact of forest fires, like prescribed fires. This technique is currently the most frequently used with the main objective of eliminating surface dead fuel (Fernandes and Botelho 2003; Agee and Skinner 2005; Espinosa et al. 2018). Although it is a useful management technique to reduce the load of fine fuel, it could mean the activation of the seed bank of some species that could lead to an increase in the continuity of the fuel, which would be the case of the species studied here. The high temperatures produced by the fire would break the dormancy of the *C. ladanifer* seeds and stimulate their germination. As well as the activation of the ground seed bank, the prescribed fires could produce favourable conditions for the establishment of the species, like the increase in light and nutrients as they eliminate the competing species for the development of the seedlings. Prescribed fires would, therefore, contribute to the elimination of the surface fuel, but could also favour the continuity of the vertical fuel by developing the undergrowth. Litter fall after fire will be delayed depending on the fire characteristics and post-fire conditions. Leaves will fall on floor only when fire would be intense enough to scorch the canopy but not so much intense to remove it completely. In the meantime, heated seeds of *C. ladanifer* stored in the soil will only be able to germinate after rainfall. It is known that in Mediterranean shrublands, post-fire germination of *C. ladanifer* is used to be gathered in the first year after fire, but when the first year after fire is dry, germination is delayed until the next 2 years (Moreno et al. 2011). Thus, since the senescence of the litter would be affected by the intensity of the prescribed fires and, therefore, by the moment when they were carried out as well as the subsequent post-fire conditions, it is essential to choose the moment and the suitable zones for the execution of prescribed fires to guarantee the effectiveness of the tool for the management of the pine stands.

5 Conclusion

Fire improves the regeneration of *C. ladanifer* through different factors acting on germination and seedling growth. This species exhibits a remarkable germination capacity once seed dormancy is broken by heat shock, reaching high germination percentages across all studied treatments. Unlike fire, leaf litter had no effect on final germination and only affected the earliest stages of seedling growth. Leaf litter hinders the growth of 2-month-old seedlings due to the physical barrier that the tiny seeds need to go through for reaching the light; however, after 4 months, when plants have grown to a larger size, litter ceases to restrict their growth, while ash promotes it by providing nutrients required for seedling development. According to these findings, although the use of prescribed fires may have the desired short-term effect of reducing fine fuel loads, they also may aid the spread of *C. ladanifer*, which would have short- and medium-term implications by increasing future fuel continuity. These collateral effects are specially pronounced in pine stands of low density, where a larger soil seed bank can be activated and the addition of low amounts of ash is especially advantageous for seedlings. However, further research based on vegetation monitoring after prescribed fires is needed to support these conclusions.

Appendix

Table 5 Mean values and standard deviation of the variables of growth [length (cm) and biomass (mg)] of the seedlings of *Cistus ladanifer*. The seeds were exposed to a heat shock of 100ºC for 10 minutes. The seeds were left to germinate under different treatments Con: control; ↑Lit: high load of litter; ↓Lit: low load of litter; ↑Ash: high load of ash; ↓Ash: low load of ash. Half of the seedlings were removed after 2 months and the growth variables related to length and biomass were measured. The same procedure was repeated after 4 months with the remaining seedlings. Different letters show the significant differences in the growth of the seedlings ($P \leq 0.05$) among the treatments according to the pairwise comparison using Bonferroni's correction and the one-way ANOVA if the data were normally distributed, or the non-parametric Kruskal-Wallis test if they were not

	Total length (cm)						Aerial length (cm)						Root length (cm)					
	2 months			4 months			2 months			4 months			2 months			4 months		
	Mean	sd		Mean	sd		Mean	sd		Mean	sd		Mean	sd		Mean	sd	
Con	9.14	2.48	a	9.62	1.6	ab	1.89	0.21	a	2.47	0.58	a	7.25	2.37	a	7.15	1.82	ab
↑Lit	6.99	2.04	b	9.12	1.94	a	2.12	0.53	ab	2.77	0.52	ab	4.87	1.78	b	6.48	1.87	a
↓Lit	8.65	2.00	ab	10.24	1.78	ab	1.99	0.27	ab	2.67	0.52	ab	6.66	2.09	ab	7.57	1.54	ab
↑Ash	9.17	2.16	a	11.34	2.78	bc	2.08	0.36	ab	3.02	0.88	ab	7.09	2.19	a	8.33	2.36	bc
↓Ash	9.97	2.44	a	12.17	2.44	c	2.22	0.24	b	3.13	0.84	b	7.75	2.39	a	9.05	2.19	c

	Total biomass (mg)						Aerial biomass (mg)						Stem biomass (mg)						Leaf biomass (mg)						Root biomass (mg)					
	2 months			4 months			2 months			4 months			2 months			4 months			2 months			4 months			2 months			4 months		
	Mean	sd		Mean	sd		Mean	sd		Mean	sd		Mean	sd		Mean	sd		Mean	sd		Mean	sd		Mean	sd		Mean	sd	
Con	1.92	0.51	ac	3.10	1.29	a	1.36	0.36	ac	2.37	1.00	a	0.33	0.13	ac	0.68	0.31	a	1.00	0.31	ac	1.66	0.80	a	0.56	0.17	a	0.73	0.32	a
↑Lit	0.83	0.41	b	2.73	1.70	a	0.65	0.28	b	2.19	1.39	a	0.12	0.14	b	0.49	0.38	a	0.40	0.23	b	1.53	1.12	a	0.25	0.09	b	0.54	0.40	a
↓Lit	1.15	0.34	b	2.98	1.51	a	0.82	0.27	b	2.33	1.19	a	0.27	0.12	a	0.66	0.34	a	0.52	0.22	b	1.64	0.91	a	0.33	0.10	b	0.65	0.36	a
↑Ash	1.74	0.58	a	6.37	3.40	b	1.23	0.43	a	5.12	2.82	b	0.35	0.13	ac	1.38	0.74	b	0.88	0.33	a	3.74	2.11	b	0.51	0.17	a	1.26	0.65	b
↓Ash	2.17	0.51	c	7.35	3.77	b	1.55	0.35	c	5.85	3.03	b	0.40	0.13	c	1.60	1.02	b	1.13	0.30	c	4.25	2.48	b	0.62	0.19	a	1.50	0.83	b

Table 6 Mean and standard deviation (sd) of the variables of growth [length (cm) and biomass (mg)] of the *Cistus ladanifer* seedlings from seeds not exposed to heat shock. The seeds were left to germinate under different treatments: Con: control; ↑Lit: high load of litter; ↓Lit: low load of litter; ↑Ash: high load of ash; ↓Ash: low load of ash. All the plants were removed after 2 months and their growth variables were measures in terms of their length and biomass

Treatment	Total length (cm)		Aerial length (cm)		Root length (cm)		Total biomass (mg)		Aerial biomass (mg)		Stem biomass (mg)		Leaf biomass (mg)		Root biomass (mg)	
	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
Con	8.51	1.72	1.48	0.41	7.03	1.46	1.19	0.50	0.83	0.38	0.19	0.08	0.64	0.31	0.36	0.15
↑Lit	6.53	2.96	1.90	0.52	4.63	3.33	0.85	0.33	0.63	0.31	0.21	0.16	0.42	0.16	0.22	0.08
↓Lit	8.84	2.82	2.08	0.17	6.77	2.77	1.14	0.27	0.78	0.20	0.24	0.06	0.55	0.15	0.35	0.09
↑Ash	9.00	1.38	1.83	0.36	7.18	1.22	1.48	0.31	1.01	0.23	0.26	0.09	0.75	0.17	0.47	0.16
↓Ash	8.23	2.61	2.02	0.47	6.21	2.83	1.77	0.84	1.26	0.60	0.31	0.16	0.95	0.47	0.50	0.27

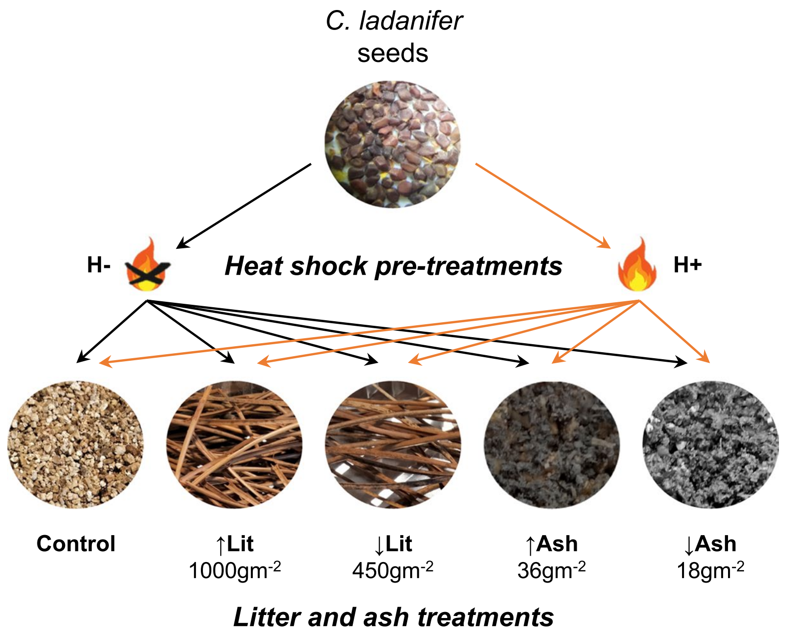

Fig. 7 Diagram of treatments applied in germination and growth experiments

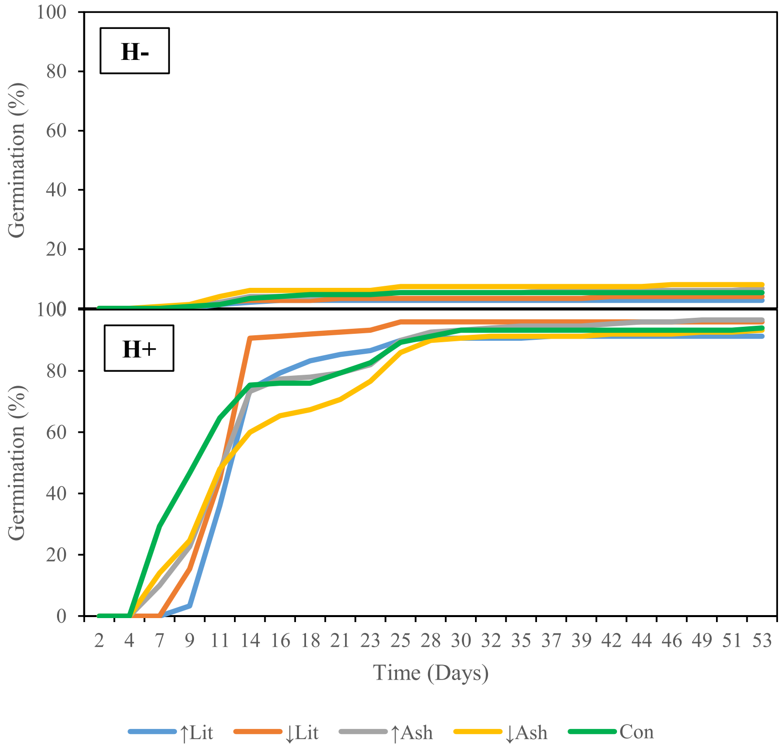

Fig. 8 Percentage of cumulative germination over the course of the experiment for H- (seeds not exposed to heat shock) and H+ (seeds exposed to heat shock). The seeds previously exposed or not to heat shock were left to germinate under five different treatments Con: control; ↑Lit: high load of litter; ↓Lit: low load of litter; ↑Ash: high load of ash; ↓Ash: low load of ash

Authors' contributions

BL conceived the study idea, and all authors designed the study. All authors carried out the experiments. PP and BL was the largest contributor to writing the manuscript. All authors read the manuscript, contributed to the writing, and approved it.

Funding

Funding was provided by Junta de Comunidades de Castilla-La Mancha, the European Regional Development Fund (ERDF) and the University of Castilla-La Mancha. This study was supported by the PRESGIF project (SBPLY/23/180225/000118).

Availability of data and materials

The datasets generated and/or analysed during the current study are available at https://zenodo.org/records/10958455

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors gave their informed consent to this publication and its content.

Competing interests

The authors declare that they have no competing interests.

Author details

[1] Departamento de Ciencias Ambientales, Universidad de Castilla-La Mancha, Av. Carlos III S/N, Toledo 45071, Spain.

Received: 1 February 2024 Accepted: 29 August 2024

References

AEMET, 2023. Weather Data from San Pablo de los Montes Station (Toledo-Spain). Available online: State Meteorological Agency of Spanish Government (AEMET), Madrid, Spain (accessed on 25 July 2023). http://www.aemet.es/

Agee JK, Skinner CN (2005) Basic principles of forest fuel reduction treatments. For Ecol Manage 211(1–2):83–96. https://doi.org/10.1016/j.foreco.2005.01.034

Arianoutsou M, Margaris N (1980) Early stages of regeneration after fire in a phryganic ecosystem (East Mediterranean). I. Regeneration by seed germination. Biologie-Ecologie Méditerraneenne. 8(3/4):119–128

Baeza MJ, Santana VM (2015) Biological significance of dead biomass retention trait in Mediterranean Basin species: an analysis between different successional niches and regeneration strategies as functional groups. Plant Biol 17(6):1196–1202. https://doi.org/10.1111/plb.12369

Baskin CC, Baskin JM (1998) Seeds: ecology, biogeography, and evolution of dormancy and germination. Elsevier

Binkley DAN, Giardina C (1998) Why do tree species affect soils? The warp and woof of tree-soil interactions. Plant-induced soil changes: Processes and feedbacks 89–106. https://doi.org/10.1007/978-94-017-2691-7_5

Bodí MB, Martin DA, Balfour VN, Santín C, Doerr SH, Pereira P, Cerdà A, Mataix-Solera J (2014) Wildland fire ash: production, composition and eco-hydro-geomorphic effects. Earth Sci Rev 130:103–127. https://doi.org/10.1016/j.earscirev.2013.12.007

Carson WP, Peterson CJ (1990) The role of litter in an old-field community: impact of litter quantity in different seasons on plant species richness and abundance. Oecologia 85:8–13. https://doi.org/10.1007/BF00317337

Comas L, Bouma T, Eissenstat D (2002) Linking root traits to potential growth rate in six temperate tree species. Oecologia 132:34–43. https://doi.org/10.1007/s00442-002-0922-8

Downes KS, Light ME, Pošta M, Kohout L, Van Staden J (2013) Comparison of germination responses of *Anigozanthos flavidus* (Haemodoraceae), *Gyrostemon racemiger* and *Gyrostemon ramulosus* (Gyrostemonaceae) to smoke-water and the smoke-derived compounds karrikinolide (KAR1) and glyceronitrile. Ann Bot 111(3):489–497. https://doi.org/10.1093/aob/mcs300

Duane A, Aquilué N, Canelles Q, Morán-Ordoñez A, De Cáceres M, Brotons L (2019) Adapting prescribed burns to future climate change in Mediterranean landscapes. Sci Total Environ 677:68–83. https://doi.org/10.1016/j.scitotenv.2019.04.348

Elvira LM, Hernando C (1989) Inflamabilidad y energía de las especies de sotobosque. Monografía INIA 68:99

Escudero A, Barrero S, Pita J M (1997) Effects of high temperatures and ash on seed germination of two Iberian pines (*Pinus nigra* ssp. *salzmannii*, *P. sylvestris* var. iberica). Annales des Sciences Forestières 54(6):553–562. EDP Sciences. https://doi.org/10.1051/forest:19970605

Espinosa J, Madrigal J, De La Cruz AC, Guijarro M, Jiménez E, Hernando C (2018) Short-term effects of prescribed burning on litterfall biomass in mixed stands of *Pinus nigra* and *Pinus pinaster* and pure stands of *Pinus nigra* in the Cuenca Mountains (Central-Eastern Spain). Sci Total Environ 618:941–951. https://doi.org/10.1016/j.scitotenv.2017.08.291

Facelli JM (1994) Multiple indirect effects of plant litter affect the establishment of woody seedlings in old fields. Ecology 75(6):1727–1735. https://doi.org/10.2307/1939632

Facelli JM, Kerrigan R (1996) Effects of ash and four types of litter on the establishment of *Eucalyptus obliqua*. Ecoscience 3(3):319–324

Facelli JM, Pickett ST (1991) Plant litter: its dynamics and effects on plant community structure. Bot Rev 57:1–32. https://doi.org/10.1007/BF02858763

Fernandes PM, Botelho HS (2003) A review of prescribed burning effectiveness in fire hazard reduction. Int J Wildland Fire 12(2):117–128. https://doi.org/10.1071/WF02042

Fernandes PM, Rigolot E (2007) The fire ecology and management of maritime pine (*Pinus pinaster* Ait.). For Ecol Manag 241(1–3):1–13. https://doi.org/10.1016/j.foreco.2007.01.010

Fernández-Guisuraga JM, Suárez-Seoane S, García-Llamas P, Calvo L (2021) Vegetation structure parameters determine high burn severity likelihood in different ecosystem types: A case study in a burned Mediterranean landscape. J Environ Manage 288:112462. https://doi.org/10.1016/j.jenvman.2021.112462

Fernández-Marcos ML (2022) Potentially toxic substances and associated risks in soils affected by wildfires: a review. Toxics 10(1):31. https://doi.org/10.3390/toxics10010031

Fowler NL (1986) Microsite requirements for germination and establishment of three grass species. American Midland Naturalist 131–145. https://doi.org/10.2307/2425843

Frazão DF, Raimundo JR, Domingues JL, Quintela-Sabarís C, Gonçalves JC, Delgado F (2018) *Cistus ladanifer* (Cistaceae): A natural resource in Mediterranean-type ecosystems. Planta 247:289–300. https://doi.org/10.1007/s00425-017-2825-2

Gallego JCA, Caro JG, Campos VH, Lobón NC (2020) Effect of leaf litter from *Cistus ladanifer* L. on the germination and growth of accompanying shrubland species. Plants 9(5):593. https://doi.org/10.3390/plants9050593

García-Plé C, Vanrell P, Morey M (1995) Litter fall and decomposition in a *Pinus halepensis* forest on Mallorca. J Veg Sci 6(1):17–22. https://doi.org/10.2307/3236251

Gómez-Vázquez I, Crecente-Campo F, Diéguez-Aranda U, Castedo-Dorado F (2013) Modelling canopy fuel variables in *Pinus pinaster* Ait. and *Pinus radiata* D. on stands in northwestern Spain. Ann for Sci 70:161–172. https://doi.org/10.1007/s13595-012-0245-9

González-Rabanal F, Casal M (1995) Effect of high temperatures and ash on germination of ten species from gorse shrubland. Vegetatio 116:123–131. https://doi.org/10.1007/BF00045303

Green SR, Kirkham MB, Clothier BE (2006) Root uptake and transpiration: From measurements and models to sustainable irrigation. Agric Water Manag 86(1–2):165–176. https://doi.org/10.1016/j.agwat.2006.06.008

Grime JP, Mason G, Curtis AV, Rodman J, Band SR (1981) A comparative study of germination characteristics in a local flora. The Journal of Ecology 1017–1059. https://doi.org/10.2307/2259651

Hamrick JL, Lee JM (1987) Effect of soil surface topography and litter cover on the germination, survival, and growth of musk thistle (*Carduus nutans*). Am J Bot 74(3):451–457. https://doi.org/10.1002/j.1537-2197.1987.tb08627.x

Hanley ME, Fenner M (1998) Pre-germination temperature and the survivorship and onward growth of Mediterranean fire-following plant species. Acta Oecologica 19(2):181–187. https://doi.org/10.1016/S1146-609X(98)80022-2

Henig-Sever N, Eshel A, Ne'eman G (1996) pH and osmotic potential of pine ash as post-fire germination inhibitors. Physiol Plant 96(1):71–76. https://doi.org/10.1111/j.1399-3054.1996.tb00185.x

Herranz JM, Ferrandis P, Martínez-Sánchez JJ (1999) Influence of heat on seed germination of nine woody Cistaceae species. Int J Wildland Fire 9(3):173–182. https://doi.org/10.1071/WF00014

Herranz JM, Ferrandis P, Copete MA, Duro EM, Zalacaín A (2006) Effect of allelopathic compounds produced by *Cistus ladanifer* on germination of 20 Mediterranean taxa. Plant Ecol 184:259–272. https://doi.org/10.1007/s11258-005-9071-6

Herrera CM (1984) Tipos morfológicos y funcionales en plantas del matorral mediterráneo del sur de España. Studia Oecologica 5(1):7–34

Herrero C, San Martin R, Bravo F (2007) Effect of heat and ash treatments on germination of *Pinus pinaster* and *Cistus laurifolius*. J Arid Environ 70(3):540–548. https://doi.org/10.1016/j.jaridenv.2006.12.027

Izhaki I, Henig-Sever N, Ne'eman G (2000) Soil seed banks in Mediterranean Aleppo pine forests: the effect of heat, cover and ash on seedling emergence. J Ecol 88(4):667–675. https://doi.org/10.1046/j.1365-2745.2000.00486.x

Jones MW, Abatzoglou JT, Veraverbeke S, Andela N, Lasslop G, Forkel M, Smith AJP, Burton C, Betts RA, van der Werf GR, Sitch S, Canadell JG, Santín C, Kolden C, Doerr SH, Le Quéré C (2022) Global and regional trends and drivers of fire under climate change. Reviews of Geophysics 60(3): e2020RG000726. https://doi.org/10.1029/2020RG000726

Keeley JE, Zedler PH (1998) Evolution of life histories in *Pinus*. In: Richardson DM (ed) Ecology and biogeography of Pinus. Cambridge University Press, United Kingdom, p 219–250

Koorem K, Price JN, Moora M (2011) Species-specific effects of woody litter on seedling emergence and growth of herbaceous plants. PLoS ONE 6(10):e26505. https://doi.org/10.1371/journal.pone.0026505

López-Santalla A, López-García M (2019) Los Incendios Forestales en España. Decenio 2006–2015. Ministerio de Agricultura Pesca y Alimentación 1–140.

Luis-Calabuig E, Tárrega R, Valbuena L (2000) Ten years of recovery of *Cistus ladanifer* after experimental disturbances. Isr J Plant Sci 48(4):271–276

Luna B, Chamorro D, Pérez B (2019) Effect of heat on seed germination and viability in species of Cistaceae. Plant Ecolog Divers 12(2):151–158. https://doi.org/10.1080/17550874.2019.1610916

Luna B, Piñas-Bonilla P, Zavala G, Pérez B (2022) Effects of light and temperature on seed germination of eight *Cistus* species. Seed Sci Res 32(2):86–93. https://doi.org/10.1017/S0960258522000149

Luna B, Piñas-Bonilla P, Zavala G, Pérez B (2023) Timing of fire during summer determines seed germination in Mediterranean Cistaceae. Fire Ecology 19(1):52. https://doi.org/10.1186/s42408-023-00210-6

Mendes P, Meireles C, Vila-Viçosa C, Musarella C, Pinto-Gomes C (2015) Best management practices to face degraded territories occupied by *Cistus ladanifer* shrublands–Portugal case study. Plant Biosystems-an International Journal Dealing with All Aspects of Plant Biology 149(3):494–502. https://doi.org/10.1080/11263504.2015.1040483

Moreno JM, Zuazua E, Pérez B, Luna B, Velasco A, Resco de Dios V (2011) Rainfall patterns after fire differentially affect the recruitment of three Mediterranean shrubs. Biogeosciences 8(12):3721–3732. https://doi.org/10.5194/bg-8-3721-2011

Moriondo M, Good P, Durao R, Bindi M, Giannakopoulos C, Corte-Real J (2006) Potential impact of climate change on fire risk in the Mediterranean area. Climate Res 31(1):85–95

Myster RW (1994) Contrasting litter effects on old field tree germination and emergence. Vegetatio 114:169–174. https://doi.org/10.1007/BF00048396

Naveh Z (1974) Effects of fire in the Mediterranean region. In: Kozlowski TT, Ahlgren CC (eds) Fire and Ecosystems. Academic Press, New York, p 401–434

Ne'eman G (1997) Regeneration of natural pine forest–review of work done after the 1989 fire in Mount Carmel. Israel International Journal of Wildland Fire 7(4):295–306. https://doi.org/10.1071/WF9970295

Ne'eman G, Lahav H, Izhaki I (1992) Spatial pattern of seedlings 1 year after fire in a Mediterranean pine forest. Oecologia 91:365–370. https://doi.org/10.1007/BF00317625

Ne'eman G, Ne'eman R, Keith DA, Whelan RJ (2009) Does post-fire plant regeneration mode affect the germination response to fire-related cues? Oecologia 159:483–492. https://doi.org/10.1007/s00442-008-1237-1

Núñez-Olivera E, Martínez-Abaigar J, Escudero JC (1996) Adaptability of leaves of *Cistus ladanifer* to widely varying environmental conditions. Funct Ecol 10:636–646. https://doi.org/10.2307/2390174

Padilla FM, Pugnaire FI (2007) Rooting depth and soil moisture control Mediterranean woody seedling survival during drought. Funct Ecol 21:489–495. https://doi.org/10.1111/j.1365-2435.2007.01267.x

Paula S, Pausas JG (2011) Root traits explain different foraging strategies between resprouting life histories. Oecologia 165(2):321–331. https://doi.org/10.1007/s00442-010-1806-y

Pausas JG, Fernández-Muñoz S (2012) Fire regime changes in the Western Mediterranean Basin: from fuel-limited to drought-driven fire regime. Clim Change 110(1–2):215–226. https://doi.org/10.1007/s10584-011-0060-6

Pausas JG, Llovet J, Rodrigo A, Vallejo R (2008) Are wildfires a disaster in the Mediterranean basin? A review. Int J Wildland Fire 17(6):713–723. https://doi.org/10.1071/WF07151

Peterson CJ, Facelli JM (1992) Contrasting germination and seedling growth of *Betula alleghaniensis* and *Rhus typhina* subjected to various amounts and types of plant litter. Am J Bot 79(11):1209–1216. https://doi.org/10.1002/j.1537-2197.1992.tb13722.x

Piñas-Bonilla, P, Pérez B, Zavala G, Luna B (2024) Seedling growth of *Cistus ladanifer* after different treatments of heat shock and addition of different loads of *Pinus pinaster* litter and ash. Version v1. Zenodo. https://zenodo.org/records/10958455. Accessed 11 Apr 2024

Reich PB, Walters MB, Tjoelker MG, Vanderklein D, Buschena C (1998) Photosynthesis and respiration rates depend on leaf and root morphology and nitrogen concentration in nine boreal tree species differing in relative growth rate. Funct Ecol 12(3):395–405. https://doi.org/10.1046/j.1365-2435.1998.00209.x

Reyes O, Casal M (1998) Germination of *Pinus pinaster*, *P. radiata* and *Eucalyptus globulus* in relation to the amount of ash produced in forest fires. Annales des Sciences Forestières 55(7):837–845. https://doi.org/10.1051/forest:19980707

Reyes O, Casal M (2004) Effects of forest fire ash on germination and early growth of four *Pinus* species. Plant Ecol 175:81–89. https://doi.org/10.1023/B:VEGE.0000048089.25497.0c

Roy J, Sonié L (1992) Germination and population dynamics of *Cistus* species in relation to fire. J App Ecol 647–655. https://doi.org/10.2307/2404472

Saura-Mas S, Paula S, Pausas JG, Lloret F (2010) Fuel loading and flammability in the Mediterranean Basin woody species with different post-fire regenerative strategies. Int J Wildland Fire 19(6):783–794. https://doi.org/10.1071/WF09066

Shaw M (1968) Factors affecting the natural regeneration of sessile oak (*Quercus petraea*) in North Wales: II. Acorn losses and germination under field conditions. J Ecol 647–660. https://doi.org/10.2307/2258097

Sosa T, Valares C, Alías JC, Chaves Lobón N (2010) Persistence of flavonoids in *Cistus ladanifer* soils. Plant Soil 337:51–63. https://doi.org/10.1007/s11104-010-0504-1

Sydes C, Grime JP (1981) Effects of tree leaf litter on herbaceous vegetation in deciduous woodland: II. An experimental investigation. J Ecol 249–262. https://doi.org/10.2307/2259829

Taboada A, Fernández-García V, Marcos E, Calvo L (2018) Interactions between large high-severity fires and salvage logging on a short return interval reduce the regrowth of fire-prone serotinous forests. For Ecol Manage 414:54–63. https://doi.org/10.1016/j.foreco.2018.02.013

Tárrega R, Luis-Calabuig E, Valbuena L (2001) Eleven years of recovery dynamic after experimental burning and cutting in two *Cistus* communities. Acta Oecologica 22(5–6):277–283. https://doi.org/10.1016/S1146-609X(01)01125-0

Thanos CA, Georghiou K, Kadis C, Pantazi C (1992) Cistaceae: a plant family with hard seeds. Israel J Plant Sci 41(4–6):251–263. https://doi.org/10.1080/0021213X.1992.10677232

Tormo J, Amat B, Cortina J (2020) Litter as a filter for germination in semi-arid *Stipa tenacissima* steppes. J Arid Environ 183:104258

Trabaud L (1995) Germination of Mediterranean *Cistus* spp and *Pinus* spp and their reoccupation of disturbed sites. Revue D'Écologie-La Terre Et La Vie 50(1):3–14

Trabaud L, Casal M (1989) Response of *Rosmarinus officinalis* seeds on different simulations of fire. Acta Oecologica (Oecologia Applicata) 10(4):355–363

Trabaud L, Lepart J (1980) Diversity and stability in garrigue ecosystems after fire. Vegetatio 43:49–57. https://doi.org/10.1007/BF00121017

Trabaud L, Renard P (1999) Do light and litter influence the recruitment of *Cistus* spp. stands? Isr J Plant Sci 47(1):1–9. https://doi.org/10.1080/07929978.1999.10676745

Valbuena L, Tarrega R, Luis E (1992) Influence of heat on seed germination of *Cistus laurifolius* and *Cistus ladanifer*. Int J Wildland Fire 2(1):15–20. https://doi.org/10.1071/WF9920015

Valera-Burgos J, Díaz-Barradas MC, Zunzunegui M (2012) Effects of *Pinus pinea* litter on seed germination and seedling performance of three Mediterranean shrub species. Plant Growth Regul 66:285–292. https://doi.org/10.1007/s10725-011-9652-4

Vasques A, Maia P, Pedro M, Santos C, Vallejo VR, Keizer JJ (2012) Germination in five shrub species of maritime pine understory—does seed provenance matter? Ann for Sci 69:499–507. https://doi.org/10.1007/s13595-012-0206-3

Vázquez-Yanes C, Orozco-Segovia A, Rincon EEA, Sánchez-Coronado ME, Huante P, Toledo JR, Barradas VL (1990) Light beneath the litter in a tropical forest: effect on seed germination. Ecology 71(5):1952–1958. https://doi.org/10.2307/1937603

Verdú M (2000) Ecological and evolutionary differences between Mediterranean seeders and resprouters. J Veg Sci 11:265–268. https://doi.org/10.2307/3236806

Waltz AE, Stoddard MT, Kalies EL, Springer JD, Huffman DW, Meador AS (2014) Effectiveness of fuel reduction treatments: assessing metrics of forest resiliency and wildfire severity after the Wallow Fire, AZ. For Ecol Manage 334:43–52. https://doi.org/10.1016/j.foreco.2014.08.026

West NE (1979) Formation, distribution and function of plant litter in desert ecosystems. In: Perry JA, Goodall DW (eds) Arid land ecosystems: structure, functioning and management. Cambridge University Press, Cambridge, p 647–659

Wittenberg L, Malkinson D (2009) Spatio-temporal perspectives of forest fires regimes in a maturing Mediterranean mixed pine landscape. Eur J Forest Res 128:297–304. https://doi.org/10.1007/s10342-009-0265-7

Xiong S, Nilsson C (1999) The effects of plant litter on vegetation: a meta-analysis. J Ecol 87(6):984–994. https://doi.org/10.1046/j.1365-2745.1999.00414.x

Publisher's Note