Post-dispersal predation of *Pinus pinaster* Aiton seeds: key factors and effects on below-ground seed bank.

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ABSTRACT

Seed bank dynamics constitute a pool of natural regeneration, a complex and key process for stand persistence. The present work analyses post-dispersal predation on a *Pinus pinaster* Ait. (Maritime Pine) below-ground seed bank. The study was carried out at the experimental site of Mata de Cuellar (Segovia-Central Spain), where an integrated analysis of Maritime Pine regeneration has been underway since 2004. Seed predation was monitored every month from 2010 to 2013. A generalized linear model was fitted using 'next spring seed density' as the response variable and seed rain, micro-site conditions and annual climate variability as explanatory variables. Seed rain, distance to the nearest stump, basal area and Walter's drought index, along with percentage cover of herbaceous species, pine needle litter and shrub species had significant positive effects. The number of neighbouring trees had a significant negative effect on seed density. These results demonstrate how seed rain, summer drought and micro-site conditions are crucial to understanding the effects of predation on the below-ground seed bank. Though greater than null, the final seed bank might be insufficient for successful natural regeneration due to high mortality caused by difficult germination and establishment conditions.

Key words: Central Spain/ Natural regeneration/ Mediterranean ecosystem/ Micro-site conditions.

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1 INTRODUCTION

Natural regeneration is a complex process driven by multiple factors and component sub-processes such as seed production, crown seed bank, seed dispersal, below-ground seed bank, germination and establishment; each of which is influenced by biotic and abiotic influences. Identifying which influences determine regeneration success is therefore particularly challenging (Price et al. 2001). Natural regeneration is also a key to stand persistence for many Mediterranean species, where ecosystems are characterized by high interannual climate variability and strong drought during summer. Environmental conditions for forests are also changing: longer, more intense droughts and predominant climate irregularity (IPCC 2007) will make natural regeneration more complex in the future.

The Maritime Pine (*Pinus pinaster* Ait.) is a Mediterranean species of great economic, ecological and scenic importance in the Western Mediterranean area, covering over four million hectares among its natural range (Ribeiro et al. 2001). Moreover *Pinus pinaster* plantations were established in the southern hemisphere (mainly in South Africa and Australia) where it can escape from plantations and spread into native ecosystems (Rouget et al 2001). It was for this reason that it is considered as one of the world's worst invasive alien species (Lowe et al. 2004). Natural regeneration of this species has been analyzed after fire (Calvo et al. 2007; Vega et al. 2009) and in natural stands (Miguel-Pérez et al. 2008; Ruano et al. 2009; González-Alday et al. 2009; Rodriguez-Garcia et al. 2011a; Rodríguez-García et al. 2011b; Del Peso et al. 2012). Though recent work in the area has provided valuable information, the lack of long-term data makes natural regeneration modelling a complex process (Bravo et al. 2012).

According to Miguel-Pérez et al. (2008), Maritime Pine reproduction is adequate to guarantee natural regeneration in the Castilian Plateau (Northern Spain). Factors that limit regeneration can affect seed once it is disseminated. The most important factors are seed predation by rodents and birds, as well as difficult conditions of Mediterranean ecosystem in the initial months of seedling establishment, characterized by a strong summer drought.

Seed bank size and dynamics could be a key component of natural regeneration, especially in Mediterranean ecosystems (Keeley 1991; Ne'eman and Izhaki, 1999; Marañón 2001). Fire is considered a perturbation inherent to Mediterranean basin forests and some species are adapted to ensure regeneration after a fire. For instance, serotinous cones remain mature seeds for dispersal after fire forming a long-term canopy seed bank (Daskalakou and Thanos 1996; Tapias et al. 2001). *Pinus pinaster* can be serotinous or non-serotinous because there is a high intraspecific variability (Tapias et al. 2004). Castilian Plateau provenance is considered as weakly serotinous (Tapias et al 2001; Tapias et al. 2004) and any fire occurred in the experimental site of the present work during at least 10 years so it is expected serotiny was not important during the study period.

Seed dormancy has been identified in delaying *Pinus pinaster* germination (Álvarez et al, 2007; Herrero et al. 2007; Lafuente-Laguna et al. 2012). In the period prior to germination, dormant seeds are vulnerable to predation by rodents, birds and insects. Seed predation has been studied for other *Pinus* species such as *Pinus pinea* L. (Manso et al. 2014) or *Pinus nigra* Arn. (Ordóñez and Retana 2004; Lucas-Borja et al. 2010) in Spain; *Pinus sylvestris* L. in Spain (Castro et al. 1999) and Scotland (Worthy et al. 2006) or *Pinus cembra* L. in Italy (Zong et al. 2010); but there is scarce knowledge about the effects of predation on the *Pinus pinaster* below-ground seed bank. Seed predation is usually analysed using the same number of seeds throughout the experiment (Castro et al. 1999; Walters et al. 2005; Lucas-Borja et al. 2010; Manso et al. 2014). In the present work, however, predation was analysed based on the seed rain at the experimental site, in order to determine the amount of seeds available to predators. In this way, seed predation analysis was better integrated with the natural regeneration process and the feeding effect typically found in these types of studies could be eliminated.

The objective of this study was to analyze the effect of predation on the *Pinus pinaster* below-ground seed bank. Seed rain was monitored for four different levels of coverage at the experimental site in Cuéllar (central Spain) during a three-year period. Seeds collected from seed rain were replaced with an equal number of seeds placed on the ground to monitor total predation. In this way the effects on below-ground seed bank were analysed based on seed rain. The experimental site was visited every month, making it possible to evaluate seed predation over time. We expected that: (1) seed predation rates would not be constant along the time being lower during summer; (2) seed predation rates would be higher when seed availability would be higher too; (3) a negative effect would be expected on below-ground seed bank near trees and stumps; (4) soil covers would affect predation rates due to the habitat preferences of predators;

(5) below-ground seed bank would be influenced by climatic variability because droughts would affect population dynamicas of potential predators. The data obtained from this study will contribute to our understanding of the impact of predation on below-ground seed bank for different levels of coverage. This in turn will help define the most appropriate forest management strategy for ensuring natural regeneration in this type of forest.

2 MATERIALS AND METHODS

2.1 Study area and experimental design

The experiment was carried out in a natural *Pinus pinaster* stand at the long-term experimental site of Mata de Cuéllar (Segovia) in central Spain (Figure 1). The site is located in a flat, sandy region characterized by a semi-arid Mediterranean climate, with a strong summer drought and a three-month period of potential frost. Mean rainfall is 610 mm and mean temperature is 11.2 ºC. The floristic community within the stand is composed of Continental Mediterranean annual species typical of central Spain (*Micropyrum tenellum* (L.) Link, *Sedum amplexicaule* DC., *Vulpia myuros* (L.) C.C. Gmelin, *Lupinus angustifolius* L.): patches of shrubs (*Lavandula pedunculata* Miller, *Helichrysum italicum* (Roth) G. Don) and isolated Stone Pine trees (*Pinus pinea*). Silviculture in the area is traditionally based on natural regeneration following a seed tree system adapted to resin production, leading to low tree density.

The experimental site consists of ten 70 x 70 m plots and was established in 2004 for an integrated analysis of Maritime Pine regeneration. Four treatments were tested (control and 3 harvest intensities: 25%, 50% and 100% reduction of the original basal area) in a split-plot design, where one plot corresponded to the control and the harvest intensities were replicated 3 times (Figure 1). The average basal area of the plots before harvest was 17.39 m^2/ha. In each plot, nine seed traps (1m x 1m) were systematically placed to analyse seed rain and 25 1m x 1m subplots were established to monitor seed germination and seedling survival. Seed rain has been collected and regeneration subplots monitored every month since 2004. To analyse seed predation, 5 nursery trays per plot (a total of 50 trays) were buried near 5 of the 9 seed traps of the plots in 2010 (Figure 1). Nursery trays had 54 square cells (5 cm x 5cm) and they were buried to use square cells as a patern. Every month, the same amount of seeds that had been collected in the seed traps was placed on the ground, one seed per cell of the nursery tray. Seeds collected in the traps were preserved and the seeds placed on the ground were obtained from “El Serranillo” National Forest Breeding Centre (http://goo.gl/7NZ6Ob).

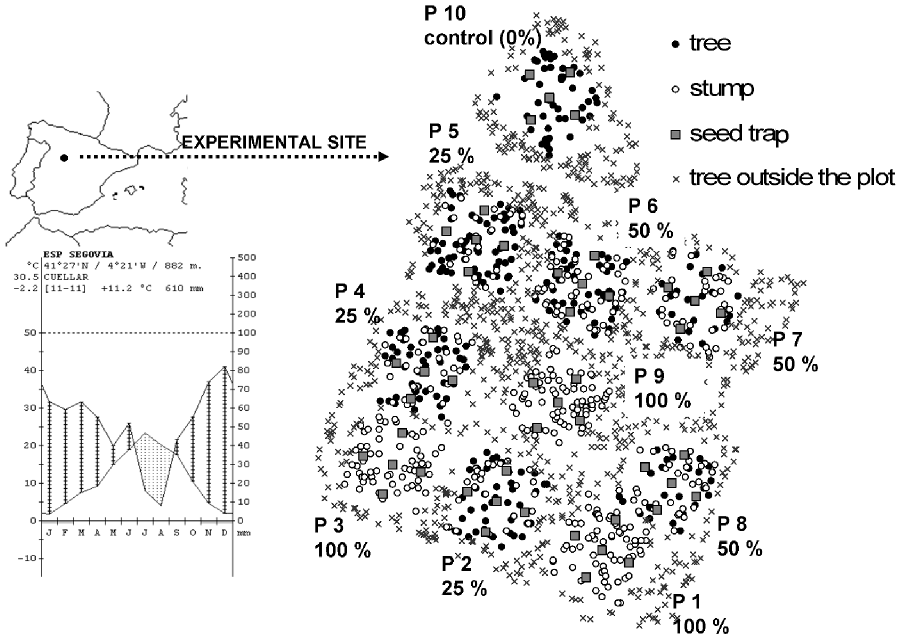

Figure 1. Location of the experimental design with a climodiagram. Position of all the trees and stumps of the experimental site and position of the seed traps and nursery trays to control predation. Harvest intensity is indicated near each plot. Climodiagram from Rivas-Martínez and Rivas-Sáenz (1996-2009).

In this way, every visit seeds collected in the seed traps was made avaliable to predators and seed predation was monitored monthly until March of 2013. During this period of time some seeds germinated and they were monitored too. Potential predators (ants, rodents and birds) were considered as a single group and not evaluated individually or by types. It was not possible to analyse isolated effects, but evidence of different types of predators was found in the nursery trays: seeds opened in the middle (birds), gnawed seeds (rodents) and missing seeds (birds, rodents and ants) (Bang and Dahlstrom 1999). Manso et al. 2014 identified potential predators of *Pinus pinea* seeds in in the same study area (Castilian Plateau) using a self-activating camera. The main predator was wood mouse (*Apodemus sylvaticus*) (80% of the recorded videos). Regarding birds, azure-winged magpie (*Cyanopica cyanus*), common raven (*Corvus corax*) and common crossbill (*Loxia curvirostra*) were identified. Moreover, during monitoring of the experimental site other species of rodents as common vole (*Microtus arvalis*) or birds like great tit (*Licania campestre*), blue tit (*Cyanistes caeruleus*), common chaffinch (*Fringilla coelebs*), eurasian nuthatch (*Sitta europaea*), eurasian jay

(*Garrulus glandarius*), common magpie (*Pica pica*) or common blackbird (*Turdus merula*) were identified by the authors so they could be considered as potential predators.

2.2 Data analysis

Seed rain and predation data were collected monthly for three years, from April 2010 to March 2013. The experimental year started in April and ended in March of the next calendar year because seed dispersal of *Pinus pinaster* begins around April and dispersed seeds germinate the following spring (Escudero et al. 2002; Del Peso et al. 2012; Lafuente-Laguna et al. 2012).

The objective of the present work was to analyse the below-ground seed density at the end of each year in order to ascertain potential below-ground seed bank for germination. Thus, the number of seeds at the end of each year was considered as the response variable. The distribution of frequencies did not fulfil the standard normality assumption, so the response variable was examined by a generalized linear model according to a Poisson distribution. All analyses were performed with the GENMOD procedure in the SAS 9.2 statistical package (SAS Institute Inc., USA) and different explanatory variables were evaluated (Table 1 and 2):

- Seed rain. The same number of seed collected from the seed traps were sown in the nursery trays, so predators had different numbers of seeds available in each nursery tray. Basic (x^2 and x^3) and inverse polynomial ($1/x+x$) seed rain transformations (Sit and Poulin-Costello 1994) were evaluated according to the functional response of predators, because intake rate does not always increase with food density in a linear fashion (Begon et al. 1988).
- Micro-site conditions: Tree and stump density. A 7.98 m radius subplot (200 m^2) was defined with each seed trap as the centre. Trees and stumps were counted in these circular subplots and basal area was also estimated.
- Micro-site conditions: Distances. Distances between the seed trap and nearest tree and stump were calculated based on coordinates taken from the extensive experimental site maps.
- Micro-site conditions: Cover. Percentage of vegetation cover in regeneration subplots was estimated for herbaceous cover (annual species), moss and lichen, pine

needle litter, mineral ground (mainly sand), shrub species (such as *Cytissus*, *Retama* or the *Halimium* genus) and woody debris (branches or pine cones).

Table 1. Characteristics of the variables: sampling size (n), mean, minimum, maximum and standard deviation. Subplot was estimated as a 7.98 m circular plot (200 m^2) considering each seed trap as the centre.

Variable	n	mean	minimum	maximum	standard deviation
Final seed density (seeds/m^2)	150	2.13	0.00	53.00	5.74
Seed rain (seeds/m^2)	150	24.69	1.00	110.00	19.47
Distance to the nearest tree (m)	50	9.77	1.13	38.76	8.75
Distance to the nearest stump (m)	50	6.54	1.63	16.23	3.48
Number of trees per subplot	50	1.00	0.00	4.00	1.01
Number of stumps per subplot	50	1.54	0.00	6.00	1.62
Basal area (m^2 per subplot)	50	0.133	0.000	0.476	0.132
Herbaceous cover (%)	50	36.00	0.00	97.00	26.09
Moss and lichen cover (%)	50	21.48	0.00	90.00	24.33
Pine needle litter cover (%)	50	23.67	0.00	95.00	26.84
Mineral ground cover (%)	50	11.11	0.00	70.00	16.55
Shrub species cover (%)	50	1.70	0.00	22.00	4.17
Woody debris cover (%)	50	12.20	0.00	70.00	15.63

Table 2. Climatic variables during considered years (year 2010: from April 2010 to March 2011; year 2011: from April 2011 to March 2012; year 2012: from April 2012 to March 2013) – (0.333 x mean daily rainfall from June to August), (Walter 1973).

Year	2010	2011	2012
Total annual precipitation (mm)	360.8	242.4	542.6
Daily mean precipitation from June to August (mm)	0.90	0.58	0.30
Daily mean annual temperature (ºC)	11.70	12.13	12.07
Daily mean temperature from June to August (mm)	20.84	20.26	21.02
Walter's drought index	20.54	20.07	20.96

- Annual climate variability. Summer drought was considered the main factor characterizing the years studied. A summer drought index was estimated according to the method proposed by Walter (1973): summer drought = (mean daily temperatures from June to August) – (0.333 x (mean daily rainfall from June to August)) (Table 2). Higher values indicate higher drought. This index has been used to analyse the effects of summer drought on the reproduction of other Mediterranean species (Espelta et al. 2008; Espelta et al. 2011).

3 RESULTS

Seed dispersal began in spring, and maximum dispersion occurred in summer (July and August in the first two years, June in the third year). Predation was low until autumn, when it significantly reduced the below-ground seed density (Figure 2). For instance, an average seed rain of approximately 40 seeds/m^2 was observed in Plot 5, where 25% of basal area had been removed, but by the end the third year the average seed density was close to 15 seeds/m^2. Some plots with lower seed rain had a higher final seed density, such as Plot 4 or Plot 7 during the third year (Figure 3).

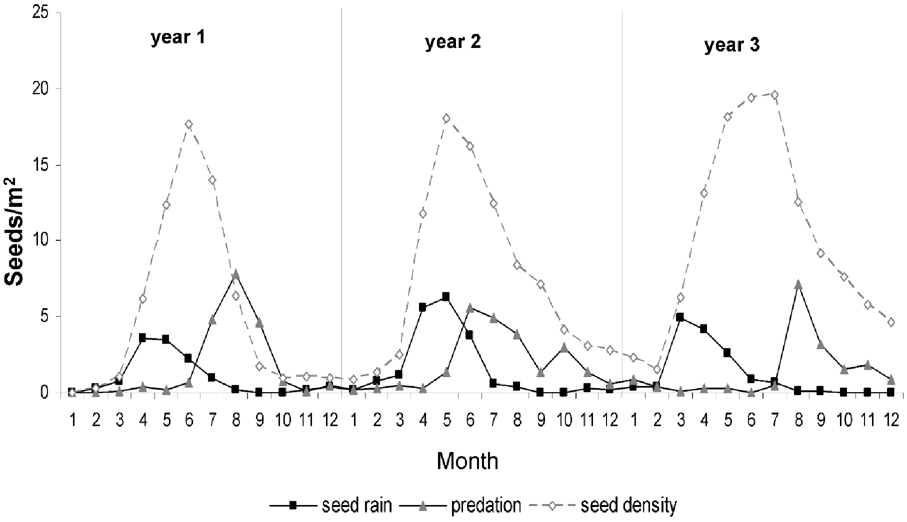

Figure 2. Mean values of seed rain, predation and seed density along the time. Month 1: April. Month 12: next year March.

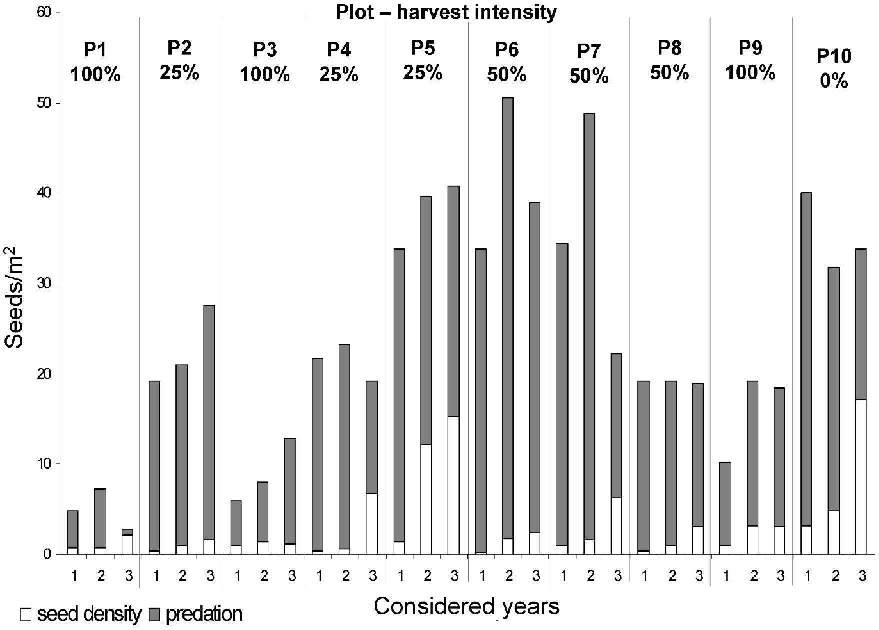

Figure 3. Mean values of seed density, predation and seed rain (total bars) for each plot (indicanting harvest intensity) and considered year (year 1: from April 2010 to March 2011; year 2: from April 2011 to March 2012; year 3: from April 2012 to March 2013).

During the monitoring of the experiment some seeds germinated and they were considered as seed density for statistical analysis. In general terms half of the seed density germinated at the end of the monitoring (Table 3). Apart from monitored seeds for predation, seed germination and seedling survival in the regeneration subplots were monitored too. Seed germination seems to be higher during 2010 and lower during 2011. It was zero every year in plots where total removal was applied but in the rest of the plots it does not seem to be low (between 3.2 and 22 germinated seeds per m^2) whereas seedling survival during the first summer was practically null (Table 4).

Table 3. Seed germination of seed density during the monitoring. Harvest intensity: percentage of harvest intensity respect basal area; Seed density: average of seed density at the end of the monitoring (seeds/m^2); germination: average of germinated seeds of seed density (seeds/m^2); Germination (%): percentage of germinated seeds respect seed density.

Plot	Harvest intensity (%)	Seed density	Germination	Germination (%)
1	100	3.8	2	52.6
2	25	3	1.4	46.7
3	100	3.6	1.8	50.0
4	25	7.8	4.6	59.0
5	25	28.8	11.2	38.9
6	50	4.4	0.6	13.6
7	50	9	5.6	62.2
8	50	4.4	2.4	54.5
9	100	7.2	3.2	44.4
10	0	25.2	12.2	48.4

Table 4. Seed germination and seedling survival in the subplots where germination and survival is monitored. G 2010: average seed germination per m^2 during 2010; G 2011: average seed germination per m^2 during 2011; G 2012: average seed germination per m^2 during 2012; Germination: average seed germination per m^2 during the study period; Survival: average seedling survival per m^2 during the first summer.

plot	Harvest intensity (%)	G 2010	G 2011	G 2012	Germination	Survival
1	100	0	0	0	0	0
2	25	13	2.2	4.2	19.4	0.2
3	100	0	0	0	0	0
4	25	3.2	0	0	3.2	0
5	25	12.6	0.2	1.4	14.2	0
6	50	7.6	0.2	1	8.8	0
7	50	6.2	0	0.4	6.6	0.2
8	50	3	0	2.8	5.8	0
9	100	0	0	0	0	0
10	0	20.2	1.2	0.6	22	1

After fitting different generalized linear models (Table 5), the following variables were selected (P-values ≤ 0.05 were considered significant) (Table 6):

- Seed rain: basic quadratic transformation
- Micro-site densities: number of trees and basal area per subplot
- Micro-site distances: distance to the nearest stump
- Micro-site covers: percent coverage of herbaceous species, of pine needle litter and of shrub species
- Annual climate variability: Walter's drought index

Akaike's Information Criterion (AIC) (Akaike 1973) was used to determine the best model from among the candidates.

Table 5. Tested models. "-": non tested variable; "ns": explanatory variable was not significant; "**": explanatory variable was significant (P-value ≤ 0.05). If there was non significant variables analysis was repeated without non significant variables. AIC: Akaike's Information Criterion.

	Tested models			
Evaluated explanatory variables	1	2	3	4
Square root of seed rain	**	**	-	-
Cube root of seed rain	-	-	**	-
Inverse polynomial of seed rain	-	-	-	**
Distance to the nearest tree	ns	ns	ns	ns
Distance to the nearest stump	**	**	**	**
Number of trees per subplot	**	**	**	**
Number of stumps per subplot	ns	ns	ns	ns
Basal area per subplot	**	-	**	**
Herbaceous cover	**	**	**	**
Moss and lichen cover	ns	ns	ns	ns
Pine needle litter cover	**	-	**	ns
Mineral ground cover	ns	ns	ns	ns
Shrub species cover	**	**	**	**
Woody debris cover	ns	ns	ns	ns
Walter's drought index	**	**	**	**
AIC	**897.59**	**908.59**	**917.63**	**969.02**

There was a positive square effect between seed rain and final seed density. Likewise, there was a positive effect between seed density, basal area per subplot and distance to the nearest stump. However, a negative effect was observed between seed density and the number of trees per subplot. Percent coverage from herbaceous species, pine needle litter and shrub species showed a significant positive effect. Finally, Walter's drought index also showed a significant positive effect (Table 6).

The Wald test was used to determine the statistical significance of each coefficient in the model. Seed rain and distance to the nearest stump had higher Wald chi-square values, while basal area per subplot and pine needle litter cover had lower Wald chi-square values (Table 6).

Table 6. Generalized linear model fitted: significant explanatory variables (basic quadratic transformation of seed rain), estimated parameters, standard error, Wald chi-square and P-value. Subplot was estimated as a 7.98 m circular plot (200 m^2) considering each seed trap as the centre. AIC (Akaike's Information Criterion: 897.59).

Explanatory variable	Estimated parameter	Standard error	Wald chi-square	P-value
Intercept	-20.1390	2.8597	49.59	<0.0001
$(\text{seed rain})^2$	0.0002	0.0000	133.79	<0.0001
Distance to the nearest stump	0.1057	0.0100	110.84	<0.0001
Number of trees per subplot	-0.5109	0.0891	32.85	<0.0001
Basal area per subplot	1.7227	0.6303	7.47	0.0063
Herbaceous cover	0.0156	0.0024	43.05	<0.0001
Pine needle litter cover	0.0066	0.0028	5.70	0.0170
Shrub species cover	0.1000	0.0117	73.67	<0.0001
Walter's drought index	0.9381	0.1389	45.61	<0.0001

4 DISCUSSION

Our results showed that predation reduced the *Pinus pinaster* below-ground seed bank, corroborating what had previously been reported for other Pine species in the Mediterranean basin (Castro et al. 1999; Ordóñez and Retana 2004; Zong et al. 2010; Lucas-Borja et al. 2010). Predation was low during the seed rain period, when temperatures were higher, and increased during autumn, as Manso et al. (2014) observed for *Pinus pinea* in the same study area (Northern Castilian plateau). However, while Manso et al. (2014) reported high predation during winter, Ordóñez and Retana (2004) did not find significant differences between sampling periods (January or May); though the interaction between sampling period and predator group (ants, rodents or birds) was significant. In the present work predation was low during winter but different hypotheses about the causes are possible. First, rodents are generally assumed to be more abundant during winter (Ordóñez and Retana 2004). However, the experimental site is located in a semi-arid Mediterranean climate with a 3-month frost period, so winters are colder than in other parts of Mediterranean Basin. Second, the present study analyses predation using actual number of seeds. The number of available seeds for predators differed among visits and plots because we considered seed rain to define seed density whereas typical experimental designs estimate an amount of seeds for each visit, regardless of the time of year. Most seed predation occurred during autumn and seed rain was almost zero in winter (average of 4 seeds/m^2 in January). Consequently, predation during winter could be lower because the number of available seeds for predators was lower too.

The objective of this study was to analyze the effect of post-dispersal seed predation on *Pinus pinaster*. To accomplish this objective seeds were placed on the ground to monitor predation. Evidences of different types of predators were found (seeds opened in the middle and gnawed seeds) but missing seeds were found too. We assumed that missing seeds were consumed but not always predators consume seeds, they can be responsibles for secondary dispersal. They could move seeds dispersing them to microsites that favor seedling establishment (Vander Wall et al. 2005, Hirsch 2012). It would be interesting to know the effect of secondary dispersal on the *Pinus pinaster* below-ground seed bank because the few missing seeds that they were not consumed by predators can play a key role in seedling recruitment (Chambers and MacMahon 1994).

Regarding germination in the subplots of the experimental site, we have not analyzed statistically these data but some aspects can be discussed. Other studies have been carried out in the same experimental site of this paper. González-Alday et al. (2008) analysed the effect of harvest intensities on *Pinus pinaster* seedlings and they observed that harvest intensities reduced the number of pine seedlings obtaining higher values in the control plots. Ruano et al. (2009) also analysed the effect of harvest intensities and water availability on *Pinus pinaster* germination and survival. In this case higher germination and survival rates were obtained where 25% of the basal area was removed and lower germination rates were obtained where total cut was applied. Moreover Rodríguez-García et al (2011a) studied the effect of overstorey structure, shrubs and soil properties on seedling emergence and survival of *Pinus pinaster* in a Mediterranean forest of central Spain. They concluded solar radiation could be the most important environmental factor and better results were obtained under closed canopy covers. During the monitoring of the present study germination rates were null in total cut plots and higher value was found in control plot, though we can not conclude significant differences between harvest plots. Regarding survival, it was practically null during the monitoring of the experiment so survival during the first summer could be the key moment of the process (Del Peso et al. 2012).

Worthy et al. (2006) modeled pre- and post-dispersal seed predation for *Pinus sylvestris* in Scotland and observed how predation rates changed during the regeneration process, from cone production through seed dispersal. However, they observed no important predation effect on below-ground seed bank; only about 25% of seeds were taken by post-dispersal predators.

Seed rain was a main explanatory variable in the present work. Our results were similar to those of Lucas-Borja et al. (2010), who analysed *Pinus nigra* seed predation in central-eastern Spain. They found less annual seed predation in masting years than in the year with lower seed rain. Regarding climate variability, instead they reported that a climatic variable (accumulated averaged maximum temperatures 20 days before survey date) influenced seed removal percentage. In the present work, Walter's drought index was found to be significant as a candidate explanatory variable, indicating the influence of annual climatic characteristics. In this case, drought might affect population dynamics of potential predators. For instance Díaz et al. (2010) observed population growth rate of *Apodemus sylvaticus* was positive during autumn-winter and there was a positive effect of rainfall three months before the normal breeding season (autumn-winter in Mediterranean ecosystems).

The micro-site conditions considered in the present work were the number of trees and stumps per subplot and distances to the nearest tree and stump, because they can serve as shelter or perch for predators (Walters et al. 2005). Manso et al. (2014) analysed *Pinus pinea* seed predation at four levels, according to relative positions of seed to trees and stumps. They found that predation differed according to the relative position of trees but not of stumps. The number of trees per subplot and the distance to the nearest stump were both significant in the present work, with a negative effect for the number of trees and a positive effect for the distance to the nearest stump. This could indicate that trees and stumps can provide shelters for rodents as well as they can provide perchs because birds can land on them. However, basal area per subplot also showed a significant positive effect, indicating that predation was lower when basal area was higher in an area of approximately 8 m around the seeds. Bigger trees can produce greater shade or shadow, making predation more difficult. It is also possible that predators such as rodents or ants differed in their microhabitat preferences, and that basal area can affect a group of predators. Hulme (1997) found that rodents avoided open areas whereas ants preferred them. Lucas-Borja et al. (2010) did not find basal area of the stand to be significant, perhaps because only three density levels were considered in that study (low: 15-20 m2/ha; medium: 25-30 m2/ha; high: 35-40 m2/ha). In the present work, basal area was included in the description of micro-site conditions.

Percent coverage from pine needle litter, herbaceous or shrub species was significant with a positive effect, indicating their potential for hiding seeds from predators. These results differ from other studies of different species and areas of the world. Castro et al. (1999) did not find differences between micro-habitats (under *Pinus sylvestris* canopy, in open interspaces between vegetation and under shrub canopy) to affect predation of *Pinus sylvestris* seeds in Spain. Hulme (1997) observed rodents to be the main seed predators of some Mediterranean species, especially beneath trees and low shrubs; whereas ants preferred open areas but their impact was low. Walters et al. (2005) analyzed *Acacia* sp seed predation in three different habitats of the African savanna: tall grass, grazing lawn and under *Acacia* tree canopies. They also observed higher predation in tall grass habitats, perhaps because this type of cover protected rodents. Potential predators or predator groups were not analyzed in the present work. Finally Nopp-Mayr et al. (2012) studied post-dispersal seed predation of *Picea abies* L. Karst, *Fagus sylvatica* L. and *Abies alba* Mill. in three macro-habitats (managed forest, small and large old-growth forest) and two micro-habitats (open areas or covered areas with ground vegetation) in Austria. The results were variable in the different macro- and micro-habitats, making interpretation more complex. This highlights the importance of defining micro-site conditions.

5 CONCLUSION

If potential seed bank is estimated from seed rain data without accounting for predation, the amount of seed available for germination the following spring is overestimated. Post-dispersal seed predation in natural *Pinus pinaster* regeneration is a sort of bottle-neck phenomenon. Though the final seed density is greater than null (at least 1 seed/m^2), it may be insufficient due to high mortality resulting from difficult germination and establishment conditions (Ruano et al. 2009; Rodriguez-Garcia et al. 2011b). Seed rain, trees and stumps around seeds, and coverage were important for providing seeds or protecting them from predators. Thus, seed rain, summer drought and micro-site conditions are keys to understanding the effects of predation on the final seed bank.

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