Geoderma 191 (2012) 80–88

Contents lists available at SciVerse ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Wildfire effects on the soil seed bank of a maritime pine stand — The importance of fire severity

P. Maia [a,*], J.G. Pausas [b], V. Arcenegui [c], C. Guerrero [c], A. Pérez-Bejarano [c], J. Mataix-Solera [c], M.E.T. Varela [a], I. Fernandes [a], E.T. Pedrosa [a], J.J. Keizer [a]

[a] *CESAM — Centre for Environmental and Marine Studies, Department Environment and Planning, University of Aveiro, Aveiro, Portugal*
[b] *CIDE — Centre for Research on Desertification, CSIC, Valencia, Spain*
[c] *UMH — University Miguel-Hernandez, Elche, Spain*

ARTICLE INFO

Article history:
Received 21 June 2011
Received in revised form 13 December 2011
Accepted 1 February 2012
Available online 3 March 2012

Keywords:
Wildfire
Soil seed bank
Pinus pinaster Ait.
Erica spp.
Calluna vulgaris

ABSTRACT

This study addressed the impacts of wildfire and, in particular, its severity on the seed bank of the litter/ash layer and the topsoil of a Mediterranean pine plantation (*Pinus pinaster* Ait.) in north-central Portugal. The study location was selected for presenting a homogeneous pine cover before the fire, on the one hand, and, on the other, heterogeneous patches with distinct degrees of damage to the pine crowns immediately after the fire. The experimental design involved the selection, from the opposite valley side, of three zones with adjacent strips of Low and High Canopy Consumption (L/HCC). Within each of these strips, a transect was laid out along which three plots were established at 10 m intervals. The same was done in the unburnt area immediately outside the fire perimeter. At each plot, samples were collected within the first two weeks after the fire to: (i) asses viable seed densities for three sampling layers, using the indirect method for a 10-month period; (ii) estimate maximum temperature reached (MTRs) at 0–3 cm depth, on the basis of Near Infrared Spectroscopy (NIR). Fire severity at the plots was further determined by verifying, in situ, pine canopy consumption (FCC) as well as by measuring the minimum diameter of remaining shrub twigs (TDI). In comparison with the unburnt area, the recently burnt area as a whole revealed a substantial increase in overall densities of viable seeds. Seed bank composition, however, varied markedly within the burnt area but this could be explained reasonably well by differential effects of the wildfire associated with its severity, in terms of the two crown consumption classes as well as the TDI index but not the MTRs. The inclusion of the litter/ash layer and the separation of two soil depths were amply justified by providing clear support for the important role of fire severity, in particular for the two principal taxa (*Calluna vulgaris* and *Erica* spp., presumably mainly *E. australis*).

1. Introduction

Fire is a key ecological and evolutionary factor in Mediterranean-type ecosystems (Pausas and Keeley, 2009), therefore many Mediterranean plant species present adaptive traits in relation to fire occurrence (Pausas et al., 2004) like the ability to resprout or to germinate after fire. Post-fire germination can be explained by heat toleration or fire related stimulation (Paula and Pausas, 2008), as well as chemical triggers related to smoke and/or ash exposition (Moreira et al., 2010). However, the role of heating regime in germination depends on the species, as high severity or the exposure to high temperatures may be lethal to the seeds of some species (Moreira et al., 2010; Trabaud, 1992). Besides laboratory experiments (e.g. Moreira et al., 2010; Rivas et al., 2006), also experimental fires, in particular through the use of thermo-couples installed in the topsoil prior to the burning (e.g. Fernández et al., 2008; Madrigal et al., 2010), can allow detailed insight in heating effects on germination. Wildfires, on the other hand, typically do not. Instead, the severity of wildfires is commonly evaluated using indices that reflect fire-induced damage to the above-ground vegetation/biomass, such as crown and litter consumption, diameter of remaining twigs, and colour of ash layer (Keeley, 2009; Otto et al., 2010; Pausas et al., 2003; Pereira et al., 2010; Pérez and Moreno, 1998; Roy et al., 2010; Úbeda et al., 2006). Recent studies, however, have found that Near Infrared (NIR) spectroscopy can give precise estimates of the maximum temperature produced in soils by heating, namely under laboratory conditions (e.g. Arcenegui et al., 2008; Guerrero et al., 2007). To the best of our knowledge, the present study is a first attempt to employ this methodology in a wildfire context, to estimate the temperatures reached in the top soil.

Although Mediterranean pine woodlands are particularly prone to wildfire occurrence (Fernandes and Rigolot, 2007), not all aspects of

---

[*] Corresponding author.
E-mail address: paula.maia@ua.pt (P. Maia).

0016-7061/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.geoderma.2012.02.001

their post-fire regeneration have received much research attention. Amongst the existing studies, Arianoutsou and Ne'eman (2000), Otto et al. (2010) and Pausas et al. (2003) were limited to the regeneration of the pine populations themselves. Three other prior studies (Calvo et al., 2003; Ferrandis et al., 1996; Valbuena et al., 2000a) did, in fact, assess post-fire recovery of all vascular plants and the role therein of the soil seed bank. However, unlike the present study, these studies did not explicitly address the impact of fire severity on seedling emergence.

The role of the litter layer as a potential source of seeds is not consensual. Litter can have negative (Rebollo et al., 2001) as well as positive effects on germination, due to protection of the seeds from predators (Deham et al., 2009) or enhanced seed longevity (Rotundo and Aguiar, 2005). Even if conditions in the litter layer itself are not favourable for germination or long-term storage, the litter layer can act as a temporary storage from where the seeds can be incorporated in the soil seed bank by the soil fauna such as earthworms (Zaller and Saxler, 2007). Moreover, the distribution of seeds along the vertical profile of the soil is not homogeneous (Bekker et al., 1998), neither is fire effect, due to the heat-minimising nature of soil (De Bano et al., 1977 *in* Ferrandis et al., 1996). Therefore, this study considered the vertical distribution of seeds in the top soil, including litter as a potential source of viable seeds, before and after the fire.

The principal aim of this study is to further the knowledge of direct effects of wildfire on the soil seed bank of Mediterranean pine woodlands. The study's specific objectives are the following: (1) to evaluate the wildfire effect on the soil seed bank, in terms of seed density and species composition, (2) to assess in which extent these effects are related to fire type, as defined by the proportion of crown consumption or (3) to more detailed measures of fire severity. The latter included an index based on the traditional measurement of the diameters of the burnt twigs and also the estimation of maximum temperature reached (MTR) on the soil using near infrared spectroscopy (NIR). By doing so, we also provided an evaluation of the different fire severity estimations.

2. Materials and methods

2.1. Study area

The study area is located near the village of Colmeal, in north-central Portugal (Fig. 1). It was burnt by a wildfire that occurred on August 24 2008, destroying in total some 70 ha of mainly Maritime Pine (*Pinus pinaster* Ait.) and eucalypt (*Eucalyptus globulus* Labill.) plantations. Within the study area, a west-facing slope covered with Maritime Pine (40°08′45.77″N, 7°59′08.22″W, elevation between 468 and 525 m a.s.l.) was selected for this study The main reasons for selecting this particular slope were (i) it constituted a rather homogeneous stand prior to the fire, as was evident from existing images available at Google Earth and was in line with the fact that it pertains to the common lands of the Arganil municipality and, as such, is being managed by the AFN, the Portuguese National Forestry Authority; (ii) the slope was only partially burnt by the 2008 wildfire, on the one hand, and, on the other, revealed clear signs of contrasting fire severities within the burnt area, namely well-defined differences in the consumption of the pine crowns that could easily be recognised from the opposite side of the valley (see also Section 2.2) According to information provided by various tree ring counts carried out following the felling of the burnt area, the pine stand was estimated to be approximately 25 years old at the time of the wildfire. The pine tree density before the fire was around 27,000 trees ha^{-1}. The understory vegetation at the unburnt part of the slope was mainly composed of the shrubs *Arbutus unedo* L., *Erica australis* L., *Calluna vulgaris* (I.) Hull, *Pterospartum tridentatum* (L.) Willk and *Phillyrea angustifolia* L. and the grasses *Agrostis curtisii*

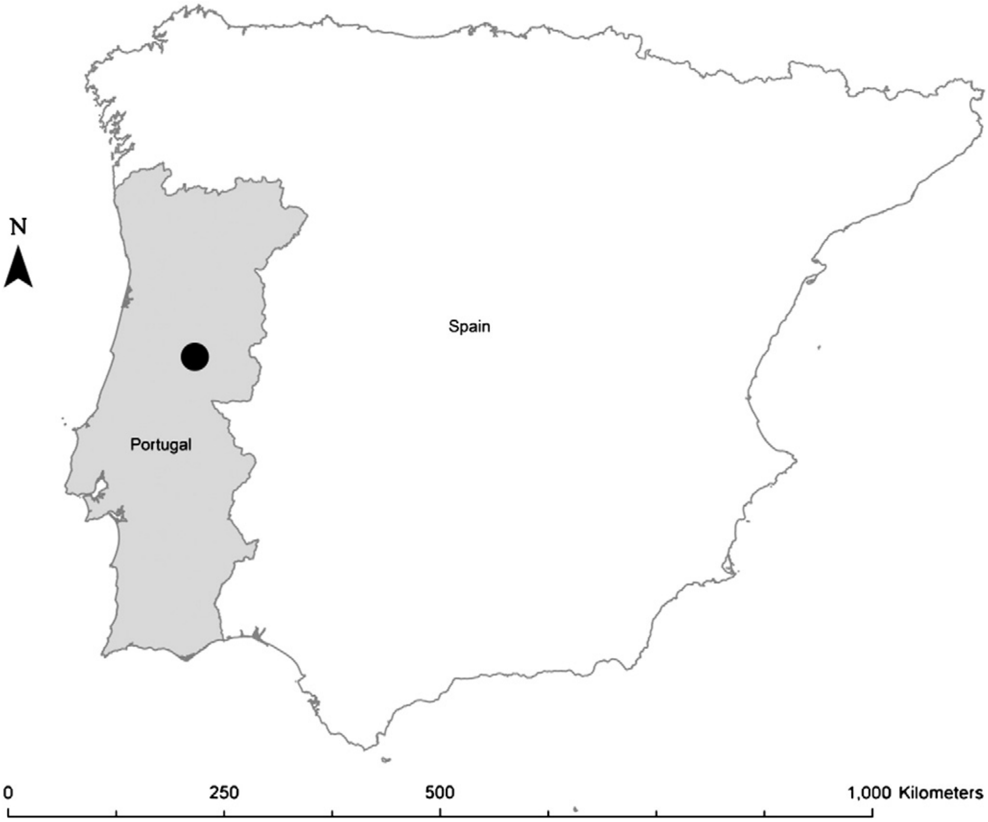

Fig. 1. Location of the study area (black circle) in the Iberian Peninsula.

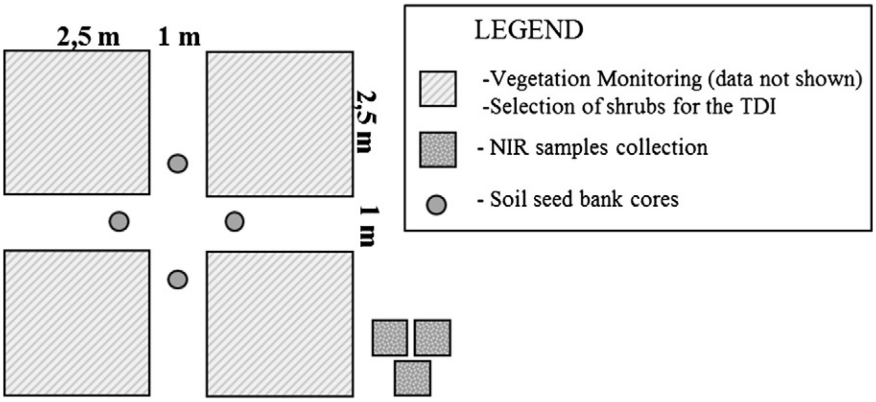

Fig. 2. Layout of the vegetation plots and of the sampling points of the seed bank cores (see Section 2.3) and the NIR samples (see Section 2.5).

Kerguélen and *Agrostis delicatula* Pourr. Ex Lapeyr (nomenclature is according to Tutin et al. (1964–1980)).

The climate of the study area can be classified as Meso-Mediterranean (Rivas-Martínez et al., 2002). The mean annual temperature is estimated to be between 10 and 12.5 °C, and the average annual rainfall between 1400 and 1600 mm (APA, 2011: 1931–1960). Two soil profiles suggested that the soils at the study site are predominantly Leptosols (WRB, 2007), and that the topsoil (0–5 cm) has a sandy loam to loam texture (sand: 29–54%; silt: 29%; clay: 16–22%) (Santos, 2010). The underlying rocks are pre-Ordovician schists of the Hercynian Massif (Ferreira, 1978).

2.2. Experimental design

Visual observation and photographs taken from the opposite side of the valley resulted in the selection of three zones where fire severity as indicated by degree of pine crown consumption had varied markedly over short distances. Dark-coloured areas were taken to reflect the (almost) complete consumption of the pine crowns, whereas light-coloured areas were interpreted to correspond to scorched crowns retaining substantial amounts of – dead – needles. The identification of adjacent areas of high and low crown consumption was done for the purpose of implementing an experimental design involving paired observations of contrasting fire severities. However, *in-situ* estimation of pine crown consumption (see underneath) obliged to a re-classification of one of the dark-coloured as well as one of the light-coloured areas, such that the experimental design continued balanced but did no longer involve paired observations.

A total of six transects with a length of approximately 30 m were laid out in the three pairs of adjacent burnt areas of apparent high and low crown consumption. An additional transect was located in the unburnt slope part nearby the fire perimeter. All transects were laid out in a perpendicular direction to the contour lines and on basically the same slope positions, so as to minimise differences in topographic and soil conditions. Along each transect, three plots of 25 m^2 were established at intervals of roughly 10 m, thus giving a total of 21 plots. In each plot, the number of totally and partially combusted crowns was counted, and the percentage of fully combusted crowns (FCC) was calculated for each plot. Based on the plot-wise FCC values, the transects in the burnt areas were classified as Low Crown Consumption (LCC) or High Crown Consumption (HCC), with FCC values ranging from 0 to 13 (mode 0) and 17 to 50 (mode 50), respectively. As mentioned above, this involved a re-classification of two transects compared to the original classification from a distance.

2.3. Soil seed bank sampling and assessment

The soil seed bank was sampled approximately two weeks after the wildfire, before the occurrence of any rain. In the centre of each plot (Fig. 2), four cores with a diameter of 6 cm were inserted into the soil to a depth of roughly 10 cm, carefully removed and transported to the laboratory, and then separated in three sub-samples: (i) ash[1] or litter (in the case of the burnt and unburnt plots, respectively); (ii) 0–3 cm soil depth and (iii) 3–6 cm soil depth. Then, the soil was sieved with a mesh of 2 mm, and the coarser particles were thoroughly inspected for pine seeds in particular.

All 252 resulting sub-samples were placed separately in aluminium trays, which had been previously perforated, on top of a 3 cm thick layer of vegetable substrate and vermiculite (1:1), and then put to germinate in a greenhouse under natural photo-period and ambient temperature conditions. The trays were regularly watered with distilled water and inspected for seedling emergence during a period of 9 months (November 2009–July 2010) and 1 additional month with no germination observed, to assure all viable seeds had emerged. 60% of the seedlings could be identified at the species level, whilst from the remaining seedlings only five could not be identified at the genus level but only at the family level as pertaining to the Asteraceae and Poaceae.

2.4. Twig Diameter Index (TDI)

Within each plot, up to 10 burnt shrubs were randomly selected, and the diameters of their 3 thinnest remaining twigs were measured. The average diameter was calculated for each plot and then re-scaled by dividing by the maximum diameter measured in this study. The resulting index, called Twig Diameter Index (TDI) ranged from 0 to 1 i.e. from low to high twig consumption and, thus, fire severity.

2.5. NIR-based estimation of the maximum temperature reached in soils

2.5.1. Model relating NIR spectra with MTR

At various points along the unburnt transect, a large (about 20 kg), composite soil sample was collected at 0–3 cm depth, carefully excluding the litter and duff layer. This soil sample was air-dried, sieved with a 2 mm mesh, and then stored in paper bags. At a later stage, a wide range of controlled heating treatments, combining different heating temperatures and durations, was carried out with aliquots of 30 g of the unburnt soil but subjecting each aliquot to a separate and single treatment. This was done in a muffle furnace, using

---

[1] "Ash" layer is, most of the times, a mixture of ashes and unburnt or partially consumed litter. For simplification purposes it is further referred to as "ash".

thermo-couples (k-type, NiCr–Ni; Testo SA, Barcelona, Spain) to measure the MTR reached at 2 cm depth inside the sample.

After cooling the aliquots to room temperature and placing them in glass Petri dishes, their NIR-spectra were measured using a FT-NIR spectro-photometer (MPA; BrukerOptik GmbH, Germany), that was equipped with a quartz beam splitter, a PbS-detector as well as an integrating macro-sample sphere and a rotating sample cup to allow for scanning large areas of the samples. The scanning was done in reflectance mode from 12,000 to 3800 cm^{-1}, which is approximately equivalent to a range of 830 to 2630 nm.

To reduce optical interference not related to the chemical composition of the sample, such as, for example, those variations caused by different sample particle sizes (Blanco and Villarroya, 2002), typical procedures of pre-processing the spectra were tested. They included no pre-processing, first derivative, second derivative, linear offset subtraction, straight line subtraction, multiplicative scatter correction, vector normalisation, min–max normalisation, and combinations thereof. Derivative treatment is considered to reduce scattering effects as well to enhance the resolution of the spectral peaks (Burns and Ciurczak, 2001). Several combinations were tested using OPUS 5.5 (BrukerOptik GmbH, Ettlingen, Germany) during calibration. First derivative and vector normalisation was the combination that produced the lowest root mean squared error of cross-validation (RMSECV), therefore being selected for the present work.

Partial least squares (PLS) regression was used to construct the model (i.e. the empirical calibration function) relating the NIR-spectra with the MTR of the unburnt soil during the various heating experiments (McCarty et al., 2002; ViscarraRossel et al., 2006). In this study, the leave-one-out cross-validation method was used. The resulting model, which provided the most satisfactory fit between the measured MTR and the MTR predictions based on the NIR-spectra was used to estimate the MTR in the burnt soils (Fig. 3).

2.5.2. Estimation of MTR of the burnt soils

At each burnt plot, a sample of the top 3 cm of the soil was collected at three nearby sampling points according to the scheme in Fig. 2. Ashes were gently but thoroughly removed from the soil surface prior to sampling, to avoid any interference with the NIR-spectra (Arcenegui et al., 2008). Sample handling and subsequent analysis with the FT-NIR spectrophotometer was basically the same as described above for the unburnt soil, except that the aliquots comprised 50 g of soil.

Each of the diffuse reflectance measurements involved 64 scans, and results of which were averaged. Furthermore, each sample was measured twice, thereby increasing the surface of soil sample scanned, and these results were also averaged. The resolution used for spectral analysis was 8 cm^{-1}. Background corrections were made before each sample scan. The time used for the spectral measurement was approximately 1 min per sample. No chemical or hazardous reagents were needed to obtain the NIR spectra.

The NIR-spectra were then used to estimate the MTR of the burnt samples, applying the model that was constructed earlier (see Section 2.5.1).

2.6. Data analysis

Statistical data analysis was carried out with IBM SPSS Statistics 19. Preference was given to non-parametric statistical tests, because of the limited number of samples and the resulting difficulties in verifying key assumptions underlying the parametric equivalents. Therefore, differences between the two fire severity classes of Low and High Crown Consumption (LCC *vs.* HCC) were tested for their significance using the Mann–Whitney *U* test.

In all tests the dependent variables considered were those related with the seed bank viability, and the independent variables were those related with fire severity.

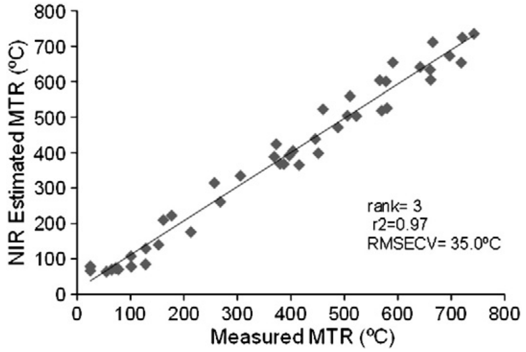

Fig. 3. Relationship between the maximum temperatures reached (MTRs) as measured in laboratory heating experiments and as predicted on the basis of the corresponding near-infrared (NIR) spectra.

3. Results

3.1. Relation of crown consumption classes with other fire severity indices

The results from the near-infrared spectroscopy (NIR) indicated that the upper, 0–3 cm soil layer suffered low to moderate heating by the wildfire, according to the criteria of Wohlgemuth et al. (2006). The maximum temperatures reached (MTRs) varied markedly between the 18 burnt plots, ranging from 53 to 125 °C. The median MTR of the LCC plots (86 °C) was slightly higher than that of the HCC plots (78 °C) but the MTRs of the two crown consumption classes were not significantly different (Mann Whitney *U*-test: $p = 0.31$), as was also easily understood from Fig. 4a.

The diameters of the burnt twigs also indicated considerable variation in fire severity amongst the 18 burnt plots, with the minimum and maximum TDI values differing an order of magnitude (0.05–0.51). Unlike in the case of the MTRs, however, the TDI values of the LCC plots were significantly lower (Mann Whitney *U*-test: $p = 0.005$), than those of the HCC plots (Fig. 4b), thus indicating a lower severity.

3.2. Seed bank composition

The overall density of viable seeds in the litter layer and the top 6 cm of the unburnt soil amounted to 1003 seeds.m^{-2}, whereas the overall density in the ash layer and burnt soil (LCC and HCC plots) was roughly 50% higher (1445 seeds.m^{-2}; Fig. 5a) This difference was mainly due to the upper soil layer (0–3 cm: 649 *vs.* 914 seeds.m^{-2}). Somewhat surprisingly, the overall densities were basically the same for the litter and ash layer (295 and 285 seeds.m^{-2}, respectively).

The overall effect of fire on germination differed markedly between LCC and HCC plots (Fig. 5b).The overall seed density of the nine LCC plots (2203 seeds.m^{-2}) was more than twice that of the unburnt plots, especially due to the two soil layers 0–3 (+120%) and the 3–6 cm (+630%). The nine HCC plots, on the other hand, revealed a negative effect of fire, as the overall density (688 seeds.m^{-2}) was about 30% lower than the unburnt plots. This reduction on viable seed density was largely due to the upper soil layer (0–3 cm: −40%). As a result of these opposite tendencies, the LCC samples contained approximately four times more viable seeds than the HCC samples. Whilst LCC densities were consistently higher than HCC densities for all three sample layers, the relative differences increased along the soil profile (ash layer: +40%; 0–3 cm: +265%; 3–6 cm: +630%).

The floristic composition of the viable seed bank consisted of 10 taxa pertaining to eight higher plant families. Seven of these families – Compositae (Compositae sp1) Caryophyllaceae (*Spergula pentandra* L.), Cistaceae (*Halimium* sp1), Fabaceae (*P. tridentatum* (L.) Willk), Pinaceae (*P. pinaster* Ait.) Poaceae (*A. curtisii* Kerguélen and *A. delicatula* Pourr. ex Lapeyr, Poacaea sp1), and Scrophulariaceae (*Anarrhinum bellidifolium*

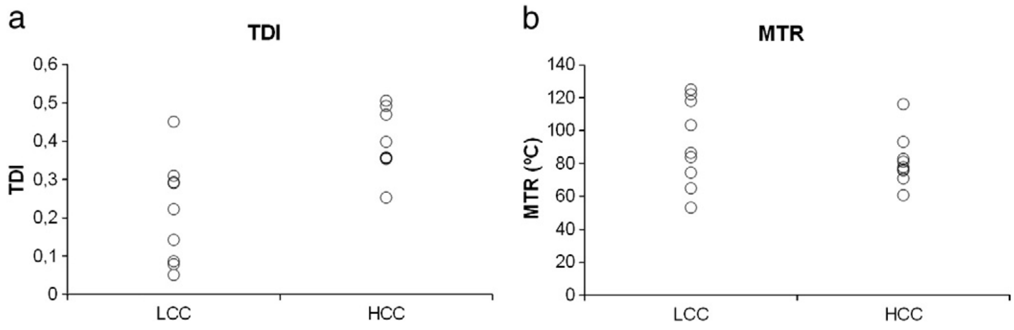

Fig. 4. Twig Diameter Index values (TDI: a) and Maximum temperatures reached (MTRs: b) for the individual plots with Low *vs.* High Crown Consumption (LCC/HCC)s.

L.) – occurred at very low frequencies, amounting to just 13% of the total number of viable seeds, and were therefore analysed together ("other spp."). The predominant family was the Ericaceae, with seeds of *C. vulgaris* (I.) Hull, *Erica umbellata* Loefl. ex L. and *E. australis* L. Due to premature death of many seedlings it was impossible to distinguish between the two *Erica* spp. for the bulk of the samples. Although *Erica* spp. was analysed here as a single taxon, *E. australis* was held to be the principal species. This opinion was based on the species' predominance in the understory of the unburnt part of the pine plantation as well as its predominance amongst the *Erica* spp. seedlings that have emerged after the wildfire and were recorded in the vegetation monitoring plots as shown in Fig. 2. The most striking difference in overall floristic seed bank composition between the unburnt and burnt (LCC and HCC) samples was the total absence of *C. vulgaris* in the former *vs.* its elevated abundance in the latter, amounting to 39% of the overall seed density (560 seeds.m^{-2}; Fig. 5a). All three sample layers contributed appreciably (>100 seeds.m^{-2}) to the overall abundance of *C. vulgaris*, even turning it into the dominant taxon of the ash layer (80%). The higher overall density of *Erica* spp. in the burnt than unburnt samples (698 *vs.* 472 seeds.m^{-2}) reflected a more complex pattern. Namely, the ash layer contained less viable *Erica* spp. seeds than the litter layer (−75%), whilst the opposite was true for the two soil layers(0–3 cm: +50%; 3–6 cm: 138 *vs.* 0 seeds.m^{-2}). The lower "other spp." on the other hand, revealed consistently lower densities in the burnt than unburnt samples for all three sample layers (−89% (ash/litter), −53% (0–3 cm soil) and −50% (3–6 cm soil)).

The above-mentioned fire-induced germination of *C. vulgaris* was evidenced by both LCC and HCC plots (Fig. 6b). In the case of the LCC plots, this inducement was clearly less pronounced for the lower soil depth (188 seeds.m^{-2}) than for the ash and upper soil layer (374 and 0 472 seeds.m^{-2}, respectively).

In the case of the HCC plots, by contrast, the *C. vulgaris* seed density was markedly higher in the lower soil layer (188 seeds.m^{-2}) than in the other two sample layers (ash: 20 seeds.m^{-2}; 0–3 cm: 20 seeds.m^{-2}). Thus, this contrast between the HCC and LCC results mainly reflected relatively low densities in the ash and upper soil layers of the HCC compared to the LCC plots.

The seed densities of *Erica* spp. suggested opposite fire effects for the two crown consumption classes in the case of the upper soil layer but not in that of the other two sample layers. Namely, the ash layer of both the LCC and HCC plots contained notably less viable *Erica* spp. seeds than the litter layer of the unburnt plots (−83 and 67%, respectively) and the lower soil layer of both the LCC and HCC plots contained viable seeds contrary to that of the unburnt plots (197 and 97 seeds.m^{-2}, respectively). The upper soil layer, on the other hand, revealed a marked increase in *Erica* spp. germination for the LCC plots (+106%) as opposed to a clear decrease for the HCC plots (−50%). The "other spp." revealed a straightforward fire effect, with both LCC and HCC plots. Even so, this reduction in viable seed densities was consistently bigger for the HCC that LCC plots (ash layer: −100 *vs.* −77%; 0–3 cm: −73 *vs.* −33%; 3–6 cm: −66 *vs.* −33%).

3.3. Seed bank spatial patterns and relation with fire severity indices

The higher overall seed density of the LCC than HCC plots was evident at the scale of the transects (Fig. 7a). The overall densities of the LCC transects varied between 1652 and 2596 seeds.m^{-2}, whereas those of the HCC transects were consistently below 1000 seeds.m^{-2} (354 to 944 seeds.m^{-2}). From the three sampling layers, however, only the upper soil layer contributed consistently to the higher densities of the LCC than HCC transects. Consistently higher densities for the LCC than HCC transects were also revealed by the two principal taxa (Fig. 7b, c). Spatial variability was especially pronounced in the case of the LCC transects. This was due to one particular transect but a different one in the case of *Erica* spp. than of *C. vulgaris*. The individual sampling layers did not tend to play a straightforward role in the consistent transect-wise differences of the two predominant taxa. An exception was the upper soil layer for *C. vulgaris*. At the transect scale, the marked spatial variability in various key seed bank

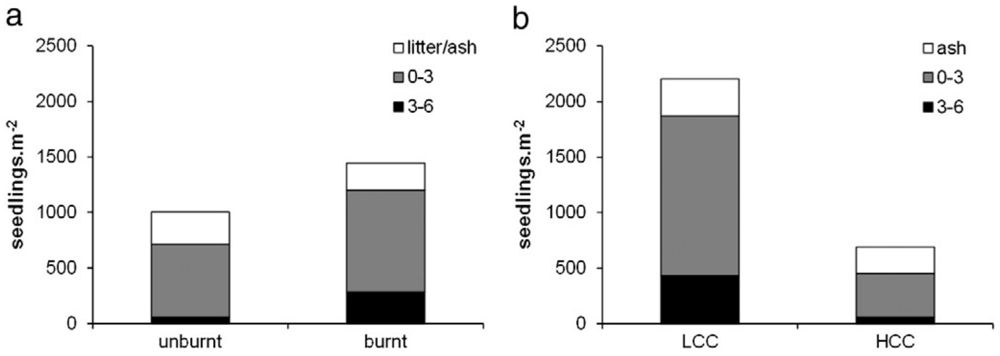

Fig. 5. Overall seed densities in the three sample layers (litter/ash, and 0–3 and 3–6 cm soil depth) for the unburnt *vs.* burnt plots (a) and for the plots with Low *vs.* High Crown Consumption (LCC/HCC: (b)).

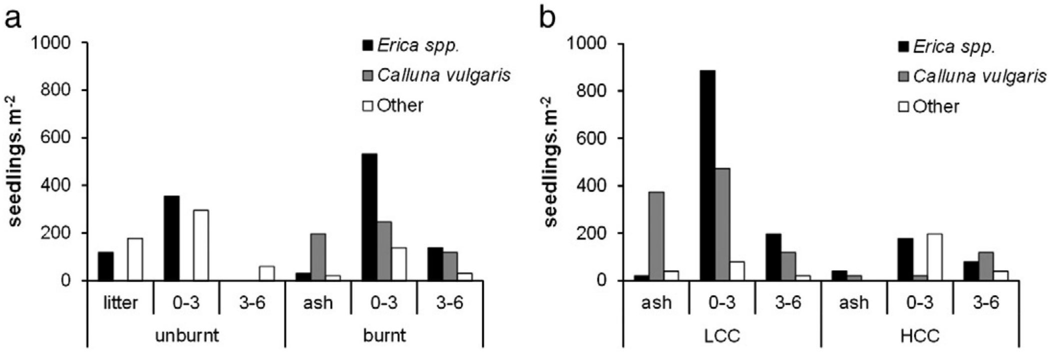

Fig. 6. Seed densities of the individual taxa in the three sample layers (litter/ash, and 0–3 and 3–6 cm soil depth) for the unburnt *vs.* burnt plots (a) and for the plots with Low *vs.* High Crown Consumption (LCC/HCC: (b)).

components within the burnt area could be well explained by the twig-base severity index. This was especially the case when analysing the seed densities of the three sample layers together. The total seed densities of the two principal taxa as well as of all taxa together (Fig. 9a) were closely associated with the transects' average TDI values, with the amounts of viable seeds decreasing with increasing TDI values and, thus, increasing fire severity. The respective Spearman's rank correlation coefficients were −0.81 for *Erica* spp. (p = 0.05), −0.89 for *C. vulgaris* (p = 0.02) and even −0.94 for all taxa (p = 0.005). These significant relationships were to an important extent due to the upper soil layer, as the Spearman coefficients for this layer separately were also relatively high (−0.71 to −0.78), even though non-significant (p = 0.07 to 0.12).

By contrast, transect-wise seed densities were poorly related to the NIR-based severity index. No significant monotonic relationships were found for either the seed densities of the three sampling layers together or the densities of the upper soil layer, for which the best association was expected as the MTRs concerned the same sampling depth (Fig. 9b). The Spearman correlation coefficients for *C. vulgaris* were remarkable in suggesting a tendency for an increase in viable seed density with increasing MTR and, thus, increasing fire severity, most markedly so in the case of the 0–3 cm soil layer (ρ = 0.62; p = 0.019).

The germination results at the plot scale provided statistical evidence in support of the earlier notions that overall and transect-wise seed densities decreased with fire severity as indicated by the two crown consumption classes. Namely, the two principal taxa both revealed significantly more viable seeds in the upper soil layer of the LCC plots than of the HCC plots (Mann Whitney *U*-test: *Erica* spp., p = 0.023; *C. vulgaris*, p = 0.014), notwithstanding the marked spatial variability amongst the LCC plots in particular (Fig. 8). The same tendency was suggested for the total seed densities of *Erica* spp. and *C. vulgaris*, with Mann Whitney *U*-test results at the border of significance (p = 0.066 and 0.063, respectively). Seed densities of all taxa together likewise hinted towards real differences between the LCC and HCC plots, especially for the upper soil layer (Mann Whitney *U*-test: p = 0.089).

Unlike at the transect scale, at the plot scale the TDI values offered little explanation for the variability in seed densities within and between the two crown consumption classes. The Spearman's rank correlation coefficients for the various combinations of taxa and sampling layers were below 0.40 (p > 0.15), with two exceptions. From these exceptions, the *C. vulgaris* densities in the upper soil layer were significantly related with the TDI values (ρ = −0.47, p = 0.045), whilst the total densities in the ash layer were almost (ρ = −0.46, p = 0.056).

The above-mentioned, poor relation of MTRs with seed densities at the transect scale extended to the individual plots. Without

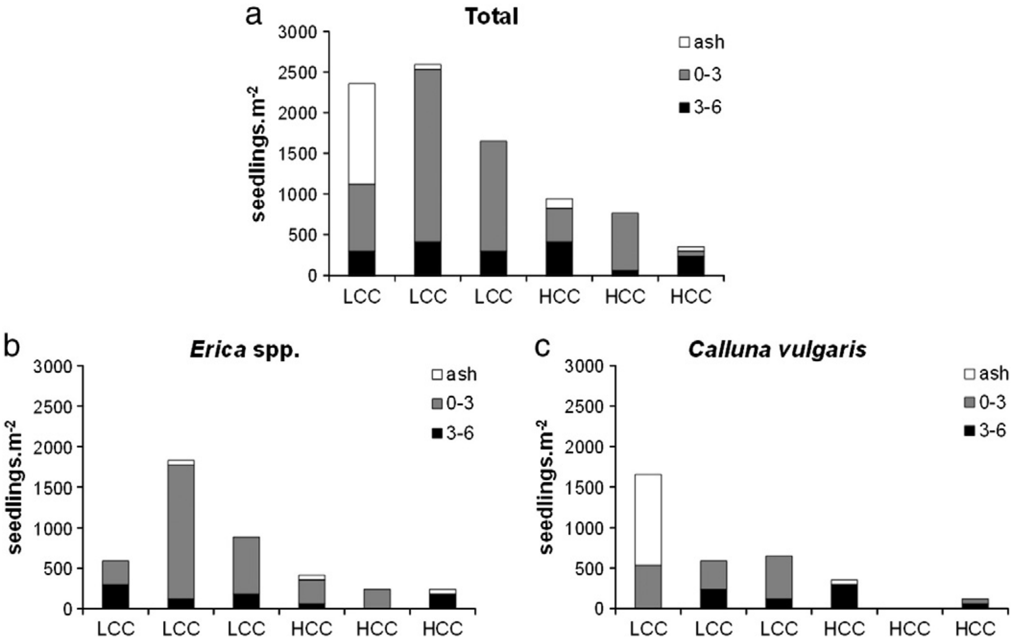

Fig. 7. Seed densities of all taxa together (a), *Erica* spp. (b) and *Calluna vulgaris* (c) densities in the three sample layers (ash; 0–3 and 3–6 cm soil depth) for the six transects, classified according to Low *vs.* High Crown Consumption and ordered according to increasing values of the Twig Diameter Index (TDI).

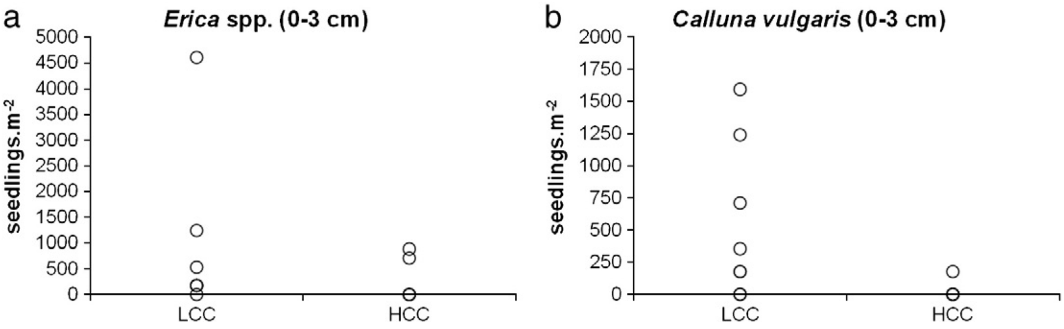

Fig. 8. Seed densities of *Erica* spp. (a) and *Calluna vulgaris* (b) in the upper soil layer (0–3 cm) for the individual plots with Low *vs.* High Crown Consumption (LCC/HCC).

exception, Spearman correlation coefficients did not differ significantly from zero, either for the seed densities of the three sampling layers together ($\rho = -0.32$ to -0.15; $p > 0.20$) or for the densities of the 0–3 cm soil depth ($\rho = -0.31$ to -0.08; $p > 0.20$).

4. Discussion

The observed difference in overall viable seed density within and outside the burnt area contrasted sharply with the findings from the prior studies that quantified pre- and post-fire seed banks of Mediterranean pine stands in a comparable manner, in particular by including all vascular plants and by employing the indirect seed bank assessment method (Ferrandis et al., 1996; Valbuena et al., 2000a). Whilst the present results indicated a marked enhancement in viable seed density after fire (+50%), the results of Ferrandis et al. (1996) and of Valbuena et al. (2000a) suggested substantial decreases of 25% and more. An explanation for these opposing effects was suggested by the present results. Namely, seed densities differed noticeably between the two fire severity classes as distinguished on the basis of the degree of consumption of the pine canopies by the flames, with the higher severity class revealing lower densities in terms of overall, transect-wise as well as (in a statistically significant manner) plot-wise figures. Ferrandis et al. (1996) explicitly referred to their wildfire as intense, due to the prevailing weather conditions, resulting in a scenario with fully charred tree crowns and understory. More importantly perhaps, their seed bank data were also consistent with a higher fire severity than in the present study. The authors found significantly less viable seeds in their upper than lower soil layer (0–2 *vs.* 2–5 cm depth; using the indirect method) and, at the same time, a large number of burnt seeds in the upper layer as opposed to none in the lower layer (using the direct method). The present results, on the other hand, revealed higher viable seed densities in the upper than lower soil layers – not only for the unburnt area but also for both crown consumption classes – and, furthermore, MTRs for the upper soil layer that were generally below 100–110 °C, i.e. temperatures that have been found to enhance the germination of at least the two *Erica* species (*E. australis*: Cruz et al., 2003; *E. umbellata*: Gonzalez-Rabanal and Casal, 1995).

Besides fire severity, other factors could contribute to the discrepancies between the present results and those of Ferrandis et al. (1996) and Valbuena et al. (2000a). Ferrandis et al. (1996) study was also done on a *P. pinaster* woodland, however the floristic composition of the seed bank was very distinct, being dominated by Cistaceae (*Cistus monspeliensis* and *C. albidus*), Fabaceae (*Trifolium glomeratum* and *T. campestre*) and *Rosmarinus officinalis*, thus, reflecting a distinctive phytosociological and biogeographical vegetation unit. This floristic difference could also be the reason for the comparatively high post-fire viable seed density in Ferrandis et al. (1996: 2200 seeds.m^{-2}), especially if their fire severity was indeed considerably higher than in the present study as argued before. Valbuena et al. (2000a), on the other hand, concerned a different pine species woodland (*Pinus sylvestris*) but with an understory as well as a seed bank that, like here, were dominated by Ericaceae (by *E. australis* and *C. vulgaris*, and by *E. australis*, respectively). Remarkably, Valbuena et al. (2000a) reported exactly the same figure for the viable seed density of the upper 5 cm of the unburnt soil as was found here for the upper 6 cm (i.e. without the litter layer: 708 seeds.m^{-2}).

Valbuena et al. (2000a) attributed the observed, negative impact of the wildfire first and foremost to high temperatures-induced damage to *E. australis* seeds, in agreement with unpublished laboratory germination trials by the same authors as well as by an experimental heathland fire (Valbuena et al., 2000b). Assuming that *E. australis* was indeed the determinant component of *Erica* spp., such a temperature-dependent effect would fit in with the present results. Namely, it could explain well: (i) the lower *Erica* spp. viable seed densities in the ash than litter layer as well as in the upper soil layer of the HCC than unburnt plots; and, at the same time, (ii) the higher densities in upper soil layer of the LCC than unburnt plots as well as in the lower soil layer of the burnt than unburnt plots. Perhaps more compelling evidence for contrasting heating effects on the germination

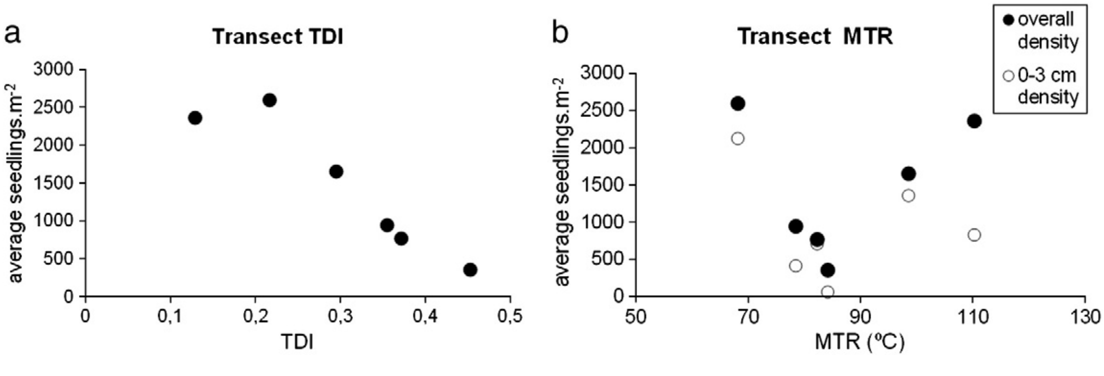

Fig. 9. Relation of the seed densities of the six transect's (overall: all sampling layers) with their average values of the Twig Diameter Index (TDI: (a)) and maximum temperature reached (MTRs: (b)).

of *Erica* spp. was provided by the statistically significant differences between the two crown consumption classes as well as the strong, quantitative relationship with the twig-based fire severity index (TDI). In accordance with Cruz et al. (2003), the threshold for temperature-induced damage to *E. australis* seeds would lie between 100 °C (enhancement) and 150 °C (no germination), at least for short exposure periods (5 min). Possibly, the importance of exposure period could explain the lack of relationships between *Erica* spp. seed densities and the NIR-based estimates of the maximum temperatures reached in the topsoil (MTRs), especially in cases like the present one in which MTRs are relatively low (Wohlgemuth et al., 2006). In addition to fire severity, genetic differences between populations resulting from distinct fire-related adaptive pressures could contribute to the observed discrepancies in the germination ecology of *E. australis* (Pausas et al., 2006).

C. vulgaris revealed a less complex response to fire than *Erica* spp. in that its germination was induced by both fire severities. Like in the case of *Erica* spp., however, there was ample evidence that this effect was markedly stronger for the plots with low than High Crown Consumption, except at the lower sampling depth. The evidence included statistically significant, inverse relationships of viable seed density with TDI values, not just at the scale of the transects but also at that of the individual plots. The latter was exceptional for this study, due to the pronounced spatial variability in seed bank composition. Valbuena et al. (2000b) also found a noticeable increase in *C. vulgaris* viable seed densities following fire but this concerned an experimental fire of a heathland. Even so, Paula et al. (2009) reported the response of *C. vulgaris* to be highly variable, covering the full range of negative to positive effects, with a possible role therein of differences between populations.

The degree of pine crown consumption proved to be a reasonably good indicator of viable seed densities immediately after wildfire. Its principal advantage – i.e. allowing a quick assessment from a distance, including from remotely sensed images – was nonetheless offset to a considerable extent by the need for *in-situ* validation (as explained in Section 2.2). The pronounced spatial variability in seed densities within the two crown consumption classes distinguished in this study could be accounted for rather well by the twig-based index (TDI), in particular for contiguous zones such as the transects but not for specific locations such as the sampling plots. Although TDI clearly provided a less subjective and more quantitative measure of fire severity than crown consumption, its time-consuming nature would seem an important limitation for widespread application under practical circumstances. The most laborious of the three fire severity indices applied here (MTR) was poorly related to the other indices as well as to viable seed densities, even at the transect scale. This could perhaps be explained by the limited significance of MTR as single and sole descriptor of heating regime, especially when MTRs are relatively low as was the case here.

5. Conclusions

The principal conclusions of this study into the direct effects of wildfire and, in particular, its severity on the seed bank of the litter/ash layer and topsoil (0–6 cm) of a Mediterranean pine stand of *P. pinaster* in north-central Portugal were the following:

1. Overall densities of viable seeds were substantially higher in the burnt area than immediately next to it but this reflected opposite effects in accordance with fire severity as distinguished based on degree of damage to the pine crowns, i.e. a germination enhancement in the case of the plots with Low Crown Consumption (LCC) *vs.* a decrease in the case of the plots with High Crown Consumption (HCC);
2. The marked variability in viable seed densities between and within the LCC and HCC severity classes could be explained well by the severity index based on the diameters of remaining twigs (TDI), in particular when aggregating the data at the scale of the transects, but not by the maximum temperatures reached in the upper soil layer (0–3 cm) (MTR) as derived from near infrared spectroscopy (NIR);
3. The densities of viable seeds in the topsoil were similar to those of prior studies in (un)burnt Mediterranean pine stands but the floristic composition of the seed bank was relatively poor and strongly dominated by Ericaceae, and, as such, compared better to that of *P. sylvestris* stand in north Spain than that of a *P. pinaster* stand in southeast Spain;
4. The viable seed densities of both predominant taxa – *Erica* spp., presumably by and large *E. australis*, and *C. vulgaris* – revealed contrasting tendencies not only with respect to fire severity in terms of LCC/HHC as well as TDIs but also in relation to the different sampling layers, plainly justifying the additional sampling efforts involved.

Acknowledgements

The PhD grant of P. Maia has been financed by FCT and QREN, the post-doc grant from M. Varela has been financed by FCT, the Leonardo da Vinci fellowships of I. Fernandes and E. Pedrosa were financed by the European Union. EROSFIRE II project, which gave the logistic support for the field campaigns, was financed by FCT. The Integrated Action that supported the NIR model development was financed by the Portuguese and Spanish governments. We also thank the AFN and GTF of Arganil, as the responsible for the management of this area, the GTF of Gois for the info on the burnt area, and the Junta de Freguesia of Colmeal for their logistic support. A special thanks to all the people who have helped in the installation of the study area, construction of the greenhouse and field sampling — Ana Vasques, Carli Mertopawiro, Roel Toonen, Diana Vieira, Robert Broekhuis, Martijn Van den Aker and José Velasco and also to two anonymous reviewers whose comments helped improving this manuscript in a significant way.

References

APA: Agência Portuguesa do Ambiente 2011. http://sniamb.apambiente.pt/webatlas/. Last time viewed November 2011.

Arcenegui, V., Guerrero, C., Mataix-Solera, J., Mataix-Beneyto, J., Zornoza, R., Morales, J., Mayoral, A.M., 2008. The presence of ash as an interference factor in the estimation of the maximum temperature reached in burned soils using near-infrared spectroscopy (NIR). Catena 74, 177–184.

Arianoutsou, M., Ne'eman, G., 2000. Post-fire regeneration of natural *Pinus halepensis* forests in the East Mediterranean Basin. In: Ne'eman, G., Trabaud, L. (Eds.), Ecology, Biogeography and Management of Mediterranean Pine Forest. Backhuys Publishers, pp. 269–290.

Bekker, R.M., Bakker, J.P., Grandin, U., Kalames, R., Milberg, P., Poschold, P., Thomson, K., Willems, J.H., 1998. Seed size, shape and vertical distribution in the soil: indicators of seed longevity. Functional Ecology 12, 834–842.

Blanco, M., Villarroya, I., 2002. NIR spectroscopy: a rapid-response analytical tool. Trends in Analytical Chemistry 21, 240–250.

Burns, D.A., Ciurczak, E.W. (Eds.), 2001. Handbook of Near-Infrared Analysis, Second edition. Marcell Dekker, New York. revised and expanded.

Calvo, L., Santalla, S., Marcos, E., Valbuena, L., Tárrega, R., Luis, E., 2003. Regeneration after wildfire in communities dominated by *Pinus pinaster*, an obligate seeder, and in others dominated by *Quercus pyrenaica*, typical resprouter. Forest Ecology and Management 184, 209–223.

Cruz, A., Pérez, B., Velasco, A., Moreno, J., 2003. Variability in seed germination at the interpopulation, intrapopulation and intraindividual levels of the shrub *Erica australis* in response to fire-related cues. Plant Ecology 169, 93–103.

De Bano, L.F., Dunn, P.H., Conrad, C.E., 1977. Fire's effect on physical and chemical properties of chaparral soils. In: Mooney, H.H., Conrad, C.E. (Eds.), Symposium on Environmental Consequences of Fire and Fuel Management in Mediterranean Ecosystems. Forest Service General Technical Report WO-3, Washington, pp. 65–74.

Deham, A., Whelan, R.J., Auld, T., 2009. Characterizing the litter in postfire environments: implications for seedling recruitment. International Journal of Plant Sciences 170 (1), 53–60.

Fernandes, P., Rigolot, E., 2007. The fire ecology of maritime pine (*Pinus pinaster* Ait.). Forest Ecology and Management 241, 1–13.

Fernández, C., Vega, J.A., Fonturbel, T., Jiménez, E., Pérez, J.R., 2008. Immediate effects of prescribed burning, chopping and clearing on runoff, infiltration and erosion in a shrubland area in Galicia (NW Spain). Land Degradation and Development 19, 502–515.

Ferrandis, P., Herranz, J.M., Martínez-Sánchez, J.J., 1996. The role of seed bank in the early stages of plant recovery after fire in a *Pinus pinaster* forest in SE Spain. International Journal of Wildland Fire 6 (1), 31–35.

Ferreira, A. de Brum., 1978. Planaltos e montanhas do norte da Beira – estudo de geomorfologia. Centro de Estudos Geográficos, Lisbon, Portugal.

Gonzalez-Rabanal, F., Casal, M., 1995. Effect of high temperatures and ash on germination of ten species from gorse shrubland. Plant Ecology 116 (2), 123–131.

Guerrero, C., Mataix-Solera, J., Arcenegui, V., Mataix-Beneyto, J., Gómez, I., 2007. Near-infrared spectroscopy to estimate the maximum temperatures reached on burned soils. Soil Science Society of America Journal 71 (3), 1029–1037.

Keeley, J.E., 2009. Fire intensity, fire severity and burn severity: a brief review and suggested usage. International Journal of Wildland Fire 18, 116–126.

Madrigal, J., Hernando, C., Guijarro, M., Vega, J.A., Fontúrbel, T., Pérez-Gorostiaga, P., 2010. Smouldering fire-induced changes in a Mediterranean soil (SE Spain): effects on germination, survival and morphological traits of 3-year-old *Pinus pinaster* Ait. Plant Ecology 208, 279–292.

McCarty, G.W., Reeves, J.B.I.I.I., Reeves, V.B., Follet, R.F., Kimble, J.M., 2002. Mid-infrared and near-infrared diffuse reflectance spectroscopy for soil carbon measurement. Soil Science Society of America Journal 66, 640–646.

Moreira, B., Tormo, J., Estrelles, E., Pausas, J.G., 2010. Disentangling the role of heat and smoke as germination cues in Mediterranean Basin flora. Annals of Botany 105, 627–635.

Otto, R., García-del-Rey, E., Muñoz, P.G., Férnandez-Palacios, J.M., 2010. The effect of fire severity on first-year establishment in a *Pinus canariensis* forest on Tenerife, Canary Islands. European Journal Research 129, 499–508.

Paula, S., Arianoutsou, M., Kazanis, D., Tavsanoglu, Ç., Lloret, F., Buhk, C., Ojeda, F., Luna, B., Moreno, J.M., Rodrigo, A., Espelta, J.M., Palacio, S., Fernández-Santos, B., Fernandes, P.M., Pausas, J.G., 2009. Fire-related traits for plant species of the Mediterranean Basin. Ecology 90, 1420.

Paula, S., Pausas, J.G., 2008. Burning seeds: germinative response to heat treatments in relation to resprouting ability. Journal of Ecology 96, 543–552.

Pausas, J.G., Keeley, J.E., 2009. A burning story: the role of fire in the history of life. BioScience 59, 593–601.

Pausas, J.G., Bradstock, R.A., Keith, D.A., Keeley, J.E., GCTE Fire Network., 2004. Plant functional traits in relation to fire in crown-fire ecosystems. Ecology 85, 1085–1100.

Pausas, J.G., Ouadah, N., Ferran, A., Gimeno, T., Vallejo, R., 2003. Fire severity and seedling establishment in *Pinus halepensis* woodlands, eastern Iberian Peninsula. Plant Ecology 169, 205–213.

Pausas, L.G., Keeley, J.E., Verdú, M., 2006. Inferring differential evolutionary processes of plant persistence traits in Northern Hemisphere Mediterranean fire-prone ecosystems. Journal of Ecology 94, 31–39.

Pereira, P., Úbeda, X., Baltrėnaitė, E., 2010. Mapping total nitrogen in ash after a wildland fire: a microplot analysis. Ekologija. 56 (3–4), 144–152.

Pérez, B., Moreno, J.M., 1998. Methods for quantifying fire severity in shrubland-fires. Plant Ecology 139, 91–101.

Rebollo, S., Pérez-Camacho, L., García-de-Juan, M.T., Benayas, J.M., Gómez-Sal, A., 2001. Recruitment in a Mediterranean annual plant community: seed bank, emergence, litter, and intra- and inter-specific interactions. Oikos 95, 485–495.

Rivas, M., Reyes, O., Casal, M., 2006. Influence of heat and smoke treatments in the germination of six leguminous shrubby species. International Journal Wildland Fire 15 (1), 73–80.

Rivas-Martínez, S., Díaz, T.E., Fernández-González, F., Izco, J., Lousã, M., Penas, A., 2002. Vascular plant communities of Spain and Portugal. Addenda to the syntaxonomical checklist of 2001. Itinera Geobotanica 15 (1), 5–432.

Rotundo, J.L., Aguiar, M.R., 2005. Litter effects on plant regeneration in arid lands: a complex balance between seed retention, seed longevity and soil-seed contact. Journal of Ecology 93, 829–838.

Roy, D.P., Boschetti, L., Maier, S.W., Smith, A.M.S., 2010. Field estimation of ash and char colour-lightness using a standard grey scale. International Journal of Wildland Fire 19, 698–704.

Santos, A.S., 2010. Efeitos dos fogos florestais na dinâmica do azoto e carbono dos solos, na região centro de Portugal. M.Sc Dissertation. University of Coimbra, Coimbra.

Trabaud, L., 1992. From the cell to the atmosphere: an introduction to interactions between fire and vegetation. In: Trabaud, L., Prodon, R. (Eds.), Fire in Mediterranean ecosystems. Ecosystems Research Report. : Commission of the European Communities., 5. CEC, Brussels, pp. 13–21.

Tutin, T.G., Heywood, V.H., Burges, D.H., Valentine, D.H., Moore, D.M., Walters, S.M., Webb, D.A., 1964–1980. Flora Europaea. Cambridge University Press, Cambridge.

Úbeda, X., Outeiro, L.R., Sala, M., 2006. Vegetation regrowth after a differential intensity forest fire in a Mediterranean environment, Northeast Spain. Land Degradation & Development 17, 429–440.

Valbuena, L., Nuñez, R., Calvo, L., 2000a. The seed bank in *Pinus* stand regeneration in NW Spain after wildfire. Web Ecology 2, 22–31.

Valbuena, L., Tárrega, R., Luis-Calabuig, E., 2000b. Seed banks of *Erica australis* and *Calluna vulgaris* in a heatland subjected to experimental fire. Journal of Vegetation Science 11, 161–166.

ViscarraRossel, R.A., Walvoort, D.J.J., McBratney, A.B., Janik, L.J., Skjemstad, J.O., 2006. Visible, near infrared, mid infrared or combined diffuse reflectance spectroscopy for simultaneous assessment of various soil properties. Geoderma 131, 59–75.

Wohlgemuth, P., Hubbert, K., Arbaugh, M., 2006. Fire and Physical Enviroment Interactions. In: Sugihara, N., Wadgtendonk, J.W., Shadffer, K.E., Fites-Kaufman, J., Thode, A.E. (Eds.), Fire in California's Ecosystems. Regents of the University of California.

WRB, 2007. http://www.fao.org/nr/land/soils/soil/wrb-soil-maps/en/. Last time viewed November 2011.

Zaller, J.G., Saxler, N., 2007. Selective vertical seed transport by earthworms: implications for the diversity of grassland ecosystems. European Journal of Soil Biology 43, S86–S91.