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Research Article

THE EFFECTS OF ASH AND BLACK CARBON (BIOCHAR) ON GERMINATION OF DIFFERENT TREE SPECIES

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ABSTRACT

Forest fires generate large amounts of ash and biochar, or black carbon (BC), that cover the soil surface, interacting with the soil's constituents and its seedbank. This study concerns reproductive ecology assessments supported by molecular characterisation to improve our understanding of the effects of fire and fire residues on the germination behaviour of 12 arboreal species with a wide geographic distribution. For this purpose, we analysed the effects of three ash and one BC concentration on the germination of *Acacia dealbata* Link, *A. longifolia* (Andrews) Willd., *A. mearnsii* De Wild., *A. melanoxylon* R. Br., *Pinus nigra* Arnold, *P. pinaster* Aiton, *P. radiata* D. Don, *P. sylvestris* L., *Quercus ilex* L., *Q. pyrenaica* Willd., *Q. robur* L., and *Q. rubra* L. Each tree species was exposed to ash and BC created from its foliage or twigs (except for *Q. rubra,* which was exposed to ash and BC of *Ulex europaeus* L.). We monitored germination percentage, the T_{50} parameter, and tracked the development of germination over time (up to 1 yr). The BC of *A. dealbata*, *P. pinaster,* and *Q. robur* was analysed by pyrolysis-gas chromatography-mass spectrome-

RESUMEN

Los incendios forestales generan gran cantidad de ceniza y biocarbones o carbón negro (CN) que cubren la superficie del suelo, interaccionando con los constituyentes del suelo y con banco de semillas. Este estudio se centra en evaluar la ecología reproductiva apoyada en la caracterización molecular para implementar nuestro conocimiento de los efectos del fuego y sus productos en el comportamiento germinativo de 12 especies de árboles con amplia distribución geográfica. Para ello, analizamos el efecto de tres concentraciones de ceniza y una de CN sobre la germinación de *Acacia dealbata* Link, *A. longifolia* (Andrews) Willd., *A. mearnsii* De Wild., *A. melanoxylon* R. Br., *Pinus nigra* Arnold, *P. pinaster* Aiton, *P. radiata* D. Don, *P. sylvestris* L., *Quercus ilex* L., *Q. pyrenaica* Willd., *Q. robur* L. y *Q. rubra* L. Cada especie arbórea fue expuesta a ceniza y CN obtenido a partir de hojas y ramas finas de la propia especie (excepto *Q. rubra* que fue expuesta a ceniza y CN de *Ulex europaeus* L.). Calculamos el porcentaje de germinación, el T_{50} y la distribución de la germinación a lo largo del tiempo (hasta 1 año). El CN de *A. dealbata*, *P. pinaster* y *Q. robur* fue analizado por pirólisis-cromatografía de gases-espetrometría de try (PY-GC-MS) to assess the molecular composition. In six species, ash inhibited the germination, while in another five species, germination was not affected by ash or by BC. In *Q. rubra,* ash and BC stimulated its germination. This stimulating effect of the BC on *Q. rubra* is likely to be related to the chemical composition of the ash and BC obtained from *Ulex* feedstock. The BC of *U. europaeus* has a very different molecular composition than the other BC samples analysed, which, together with other factors, probably allowed for its germination stimulating effects.

masas (PI-CG-EM) para determinar su composición molecular. En seis especies, la ceniza inhibió la germinación mientras que en otras cinco especies la germinación no fue modificada ni por la ceniza ni por CN. En *Q. rubra* la ceniza y el CN estimularon su germinación. Este efecto estimulador del CN sobre *Q. rubra* parece estar relacionado con la composición química de la ceniza y el CN obtenido de *Ulex*. El CN de *U. europaeus* tiene una composición molecular muy diferente de las otras muestras de carbón analizadas, lo cual junto con otros factores, probablemente permitió sus efectos estimuladores.

Keywords: *Acacia,* ash, black carbon, germination, *Pinus, Quercus*

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INTRODUCTION

Fire is and has been an important ecological and evolutionary factor in world ecosystems for millions of years, perhaps since terrestrial vegetation existed (Trabaud 1981, Pausas 2004). Nowadays, forest fires are a serious environmental problem with millions of hectares burnt every year, affecting many human beings, destroying ecosystems, and contributing to climate change (Pausas 2004). Wildfires can decrease or completely eliminate plant cover, thereby altering rainfall interception, evapotranspiration rates, and hydrological surface processes and cycles (Cerdà and Doerr 2008) through complex interactions that generally culminate into increased erosion rates. Moreover, severe burning can affect a wide range of soil properties including nutrient availability, pH, organic matter content, texture, and structure (Certini 2005).

Among the direct consequences of wildfires is the formation of ash and black carbon (BC) layers that (1) contribute to the modification of the aforementioned physical and chemical soil properties, and (2) alter germination behaviour (rate, time, etc.) of the seeds of many species (Reyes and Casal 1998). These seeds can be present in the soil's pre-fire seedbank or can originate from the seed rain after the fire. Kemball *et al.* (2010) demonstrated that the type of ash influences the germination percentage of tree species, and Solaiman *et al.* (2012) showed that the type and quantity of BC influences the germination rate.

The solid organic residue of incomplete combustion known as BC includes materials such as char, charcoal, soot, and pyrogenic graphite (Knicker 2011). Black C has recently found a new use in agronomy as biochar (e.g., Lehmann *et al.* 2006). BC can, but does not always, improve soil water retention capacity, increase the pH, enhance aggregate stability, reduce leaching, and generally increase soil productivity, while it also has a potential for climate change mitigation through C sequestration (Schmidt and Noack 2000, Masiello 2004, Jeffery *et al.* 2011). However, the effects of BC on germination and their relation to BC molecular composition are poorly understood. Therefore, laboratory studies on the alteration of seed behaviour in the presence of BC are of great interest.

Acacia, *Pinus,* and *Quercus* are plant genera that provide much forest cover and biomass in fire-prone ecosystems around the world. For the present study of the effects of ash and BC on seed germination parameters, we selected four species from each of these genera: *Acacia dealbata* Link, *A. longifolia* (Andrews) Willd., *A. mearnsii* De Wild., *A. melanoxylon* R. Br., *Pinus nigra* Arnold*, P. pinaster* Aiton, *P. radiata* D. Don, *P. sylvestris* L.*, Quercus ilex* L., *Q. pyrenaica* Willd., *Q. robur* L., and *Q. rubra* L. Apart from their wide distribution, these species are very important from ecological, silvicultural, and cultural viewpoints. The four species of *Acacia* that were studied are native to different regions of Australia (López-González 2006), but have anthropogenically expanded worldwide through ornamental use and subsequent invasive potential. Also, *A. mearnsii* is one of the most dangerous invasive species in the world (Invasive Species Specialist Group 2014). Many species of *Acacia* generally have low natural germination rates that increase after a fire event (Mucunguzi and Oryem-Origa 1996, Arán *et al*. 2013). The genus *Pinus* is distributed mainly in the temperate regions of the Northern Hemisphere: *P. nigra* and *P. pinaster* are native to the Mediterranean Basin, *P. radiata* is native to three different areas of central-coastal California, and *P. sylvestris* forms extensive forests in Europe and Asia (Richardson and Rundel 1998). These four members of the genus *Pinus*, *P. radiata* in particular, have expanded significantly in timber plantations (Kral 1993, Lavery and Read in Richardson and Rundel 1998). The *Pinus* species usually have aerial seed banks that protect the seeds during a fire and are released afterwards (Reyes and Casal 2002). Thermal shock does not stimulate their germination (Reyes and Casal 1995).

The distribution of *Quercus* genera covers all of Europe, and *Q. robur* is a species that can be found forming extensive natural forests from the north of Norway to the south of Sicily, and from Ireland to the Balkan, Ural, and Caucasus mountains. *Q. pyrenaica* is a Mediterranean species with a reduced area of natural growth, extending through southern France, the Iberian Peninsula, northwest Morocco, and has also been cited in north Italy. The populations of *Q. ilex* are a dominant component of many sclerophyllous forests that at one time dominated vast areas of the Mediterranean region. The geographic distribution of this species is centered on the Mediterranean Basin (Castroviejo *et al.* 1999). *Q. rubra* is native to the eastern USA and Canada. It is currently grown in many regions of the world, mainly for timber production. In comparison with the genera of *Acacia* and *Pinus*, the *Quercus* species produce smaller amounts of seeds that are comparably large in size, and with higher natural germination rates (Reyes and Casal 2006). They do not contain aerial seed banks but seedling banks instead. Aerial parts of seedlings may be top-killed by fire, but can resprout if the root system is sufficiently developed. High temperatures do not enhance the germination of *Quercus* (Valbuena and Tárrega 1998) and damage the seedlings.

Soil humidity is one of the climatic variables with strong influence on the emergence and establishment of seedlings (Classen *et al.* 2010). During a fire, soil humidity is reduced, but the ash and BC layer reduces the evaporation afterwards. Despite the likely importance of ash and BC from wildfires on germination behaviour, there are few studies on the subject (González-Rabanal and Casal 1995; Reyes and Casal 1998, 2004; Kemball *et al.* 2006, 2010) and the results are highly variable. We undertook laboratory experiments using the above species incubated in ash and BC preparations.

The hypothesis is that the fire agents (ash and BC) will stimulate, inhibit and accelerate, or delay the germination behaviour of the studied species.

METHODS

The biological material used in this study were seeds of *A. dealbata, A. longifolia, A. mearnsii, A. melanoxylon, P. nigra, P. pinaster, P. radiata, P. sylvestris, Q. ilex, Q. pyrenaica, Q. robur,* and *Q. rubra*. The seeds of *Acacia* and *Quercus* were handpicked in natural populations and in plantations of the northwest of the Iberian Peninsula during the seed dispersal season (*Acacia* in spring and *Quercus* in autumn). The seeds of *Quercus* were stored at 4 °C until the beginning of the experiments (between 1 and 2 months). Seeds from four *Pinus* species were obtained from different parts of the Iberian Peninsula through the seed collection service of DGCONA (Dirección General de Conservación de la Naturaleza). We did not apply stratification because the seeds of the Spanish populations of these species do not require such treatment in order to germinate (Reyes and Casal 1995, Reyes and Casal 2006, Arán *et al.* 2013) and we did not scarify the seeds in order to focus on the effects of ash and BC.

The ash and BC were obtained by burning leaves and fine branches in a combustion stove for 20 minutes. When possible, we used ash and BC from their corresponding feedstocks, but in case such materials were not available, we selected shrub species from their understory, and we did not perform BC treatments in *Pinus* species (Table 1). Gorse (*Ulex europaeus*) was chosen as the substitute ash or a BC treatment as it is geographically widespread and especially abundant in the fire-prone shrublands and woodlands of northwest Spain. After the combustion reaction, the ash (between 0.4 mm and 1 mm diameter) and BC (>1 mm diameter) were separated by sieving.

The BC samples from selected species of each genus (*Q. ilex*, *P. pinaster* and *A. dealbata*, thought to be representative of their respective genera) and *U. europaeus* were analysed by pyrolysis-gas chromatography-mass spectrometry (PY-GC-MS) to assess their molecular properties, using an Agilent 6890 gas chromatograph, Agilent 5975 mass spectrometer (Agilent Technologies, Santa Clara, California, USA) and CDS Pyroprobe 5000 (CDS Analytical, Oxford, Pennsylvania, USA). The pyrolysis temperature was 750 ºC for 10 seconds. Main peaks were quantified according to characteristic fragment ions. A detailed de-

Table 1. Applied treatments to each species and ash and BC (Black C) sources used.

		Ash treatments			
Species	**Ash and BC source**	**Ash-Low**	**Ash-Medium**	**Ash-High**	**BC treatments**
A. dealbata	*A. dealbata*	+	+	+	+
A. longifolia	*A. longifolia*	+	+	+	+
A. mearnsii	*A. mearnsii*	+	+	+	+
A. melanoxylon	*U. europaeus*	+	+	+	
P. nigra	shrubs*	+	+	+	
P. pinaster	shrubs*	+	+	+	
P. radiata	shrubs*	+	+	+	
P. sylvestris	shrubs*	+	+	+	
Q. ilex	*Q. ilex*		+		+
Q. pyrenaica	*Q. pyrenaica*		+		+
Q. robur	*Q. robur*		+		+
Q. rubra	*U. europaeus*		+		+

* Shrubs: *Erica australis* L., *Erica cinerea* L., *Cytisus scoparius* (L.) Link, *Genista tridentata* L. (SY = *Chamaespartium tridentatum* [L.] P.E. Gibbs), and *Halimium alyssoides* (Lam.) C. Koch. After burning, the ashes of these shrubs were mixed.

scription of the technique and its application to BC is provided in Kaal *et al.* (2012), which includes a description of the gas chromatographic program used. Even though *Pinus*-derived BC was not studied here, we analysed BC of *P. pinaster* for the sake of completeness and future studies.

The treatments performed were: Control, Ash-Low, Ash-Medium, Ash-High, and BC. Five replicates of 25 seeds were used for each species and each treatment. *Acacia* and *Pinus* seeds were placed on double-thickness filter paper in 9 cm diameter Petri dishes, and *Quercus* seeds were placed on perlite in 12 cm × 20 cm trays. The filter paper and perlite do not interfere in the germination response. The amount of ash used in the different treatments was based on the quantities of ash per hectare measured by Soto *et al.* (1997) in a fire of moderate intensity: Ash-Low: 0.25 g (3.93 kg ha^{-1}), Ash-Medium 0.50 g (7.86 kg ha^{-1}), and Ash-High 1 g (15.72 kg ha^{-1}). The ash and BC were placed in the trays and Petri dishes in which the seeds were deposited. The BC treatment was performed by incubating the *Quercus* seeds sown in 1.0 g BC per tray (41.5 g m^{-2} of BC), which is within the range observed for forest fires by Ohlson and Tryterud (2000), and *Acacia* and *Pinus* species in 0.26 g of BC per Petri dish, an adequate amount considering the size of the Petri dishes. Initially, 10 ml of distilled water was added to the Petri dishes, with additional water being applied periodically (at the same time as the determination of germination parameters) to keep the seeds moist (assuring that at least one-third of its surface was in contact with water). Germination was counted every Monday, Wednesday, and Friday during the germination period of each species. A seed was considered to have germinated when its radicle had extended beyond the teguments by at least 1 mm (Côme 1970). The experiments took place spanning a period of several years and some treatments were not applied to all species (Table 1). The data obtained were used to calculate the germination percentage, the T_{50} rate (the time required to reach 50% of germination), and the distribution of germination time.

For statistical analysis, using SPSS 15.0 (IBM SPSS Statistics, Armonk, New York, USA), we used the SQRT [ACOS (germination percentage ÷ 100)] transformation on the percentage data. First, the species and treatment factors were analysed using two one-way ANOVAs: one for germination percentage data and the other for the T_{50} data. Significant interactions between these two fixed factors were observed in both ANOVAs, which implied that the main effects could not be interpreted (Underwood 1997, Quinn and Keough 2002). Then one-way ANOVAs were performed to analyse the effect of the "treatment" factor in each species separately. The level of significance (0.05) was adjusted following Benjamini and Yekutieli (2001) to 0.1625 to reduce the Type I error when several analyses are made to study the same hypotheses. In those analyses in which significant differences between the treatments were shown, the corresponding Duncan tests were performed to determine which treatments caused the differences detected. For two species (*A. mearnsii* and *P. radiata*), *post hoc* T_{50} tests were not undertaken since at least one treatment had less than two cases.

RESULTS

Molecular Composition of Black Carbon

All BC samples produced significant amounts of monocyclic (benzene, toluene, C_2-benzenes, C_3-benzenes) and polycyclic (indenes, naphthalenes, fluorene, anthracene, phenanthrene, biphenyl) aromatic hydrocarbons (MAHs and PAHs) (Figure 1). These compounds, together with the N-containing pyrolysis product benzonitrile, represent strongly charred material. The *Quercus* and *Pinus* BC pyrolysates consisted almost completely of these compounds (92% and 87% of

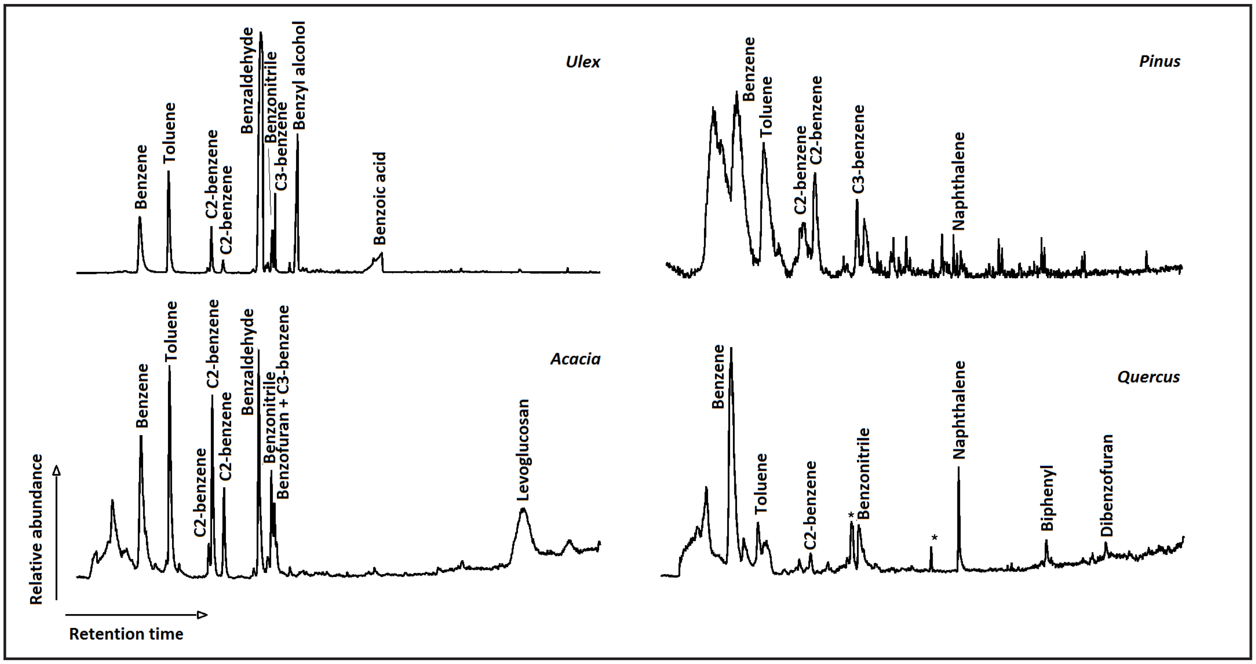

Figure 1. Total ion current chromatograms after pyrolysis-GC-MS of the black carbon samples analysed. Asterisks correspond to peaks from analytical contamination (column bleed).

total quantified peak area [TQPA], respectively). The *Acacia* BC produced, apart from the aforementioned MAHs, PAHs, and benzonitrile (63 % of TQPA), significant peaks of benzaldehyde (11 %) and levoglucosan (21 %). Considering the absence of other polysaccharide markers that would have been produced from intact cellulose, this levoglucosan probably existed as trapped volatiles in micropores. The *Ulex* BC produced only 36 % MAHs, PAHs, and benzonitrile combined, in addition to large amounts of benzaldehyde (45 %), benzyl alcohol (11 %), and benzoic acid (6 %). The extraordinarily large proportion of benzaldehyde probably originated from dehydration of the corresponding benzyl alcohol during pyrolysis. The benzene:toluene and naphthalene:C_1-naphthalenes ratios (Kaal and Rumpel 2009) were much higher for the *Quercus* BC (5.4 and 54, respectively) than for the *Acacia* (0.7 and 4), *Ulex* (0.7 and 9) and *Pinus* (1.0 and 5.1). These ratios were considered indicative of the abundance of alkyl-based cross bridges between aromatic groups and therefore a reverse indication of the degree of aromatic polycondensation.

Germination Percentage

The Control germination percentages varied widely between species (Figure 2). The ANOVA applied to the 12 species detected highly significant differences among species ($P < 0.001$) and among treatments ($P < 0.001$). In general, high germination rates, between 42 % and 97 %, were found for the species of the genera *Pinus* and *Quercus*, while low rates, between 2 % and 18 %, were found for the species of *Acacia*, except for *A. melanoxylon,* which reached 54 %. However, as the interaction between species and treatment also turned out to be significant ($P < 0.001$), we applied an additional ANOVA to the data of each species separately. Three different germination behaviours were obtained:

1) No significant differences among treatments were detected in *A. mearnsii*, *A. melanoxylon*, *Q. ilex*, *Q. pyrenaica,* and *Q. robur*;
2) Germination was significantly inhibited by the three ash treatments ($P < 0.005$) in *A. dealbata* and by the Ash-Medium

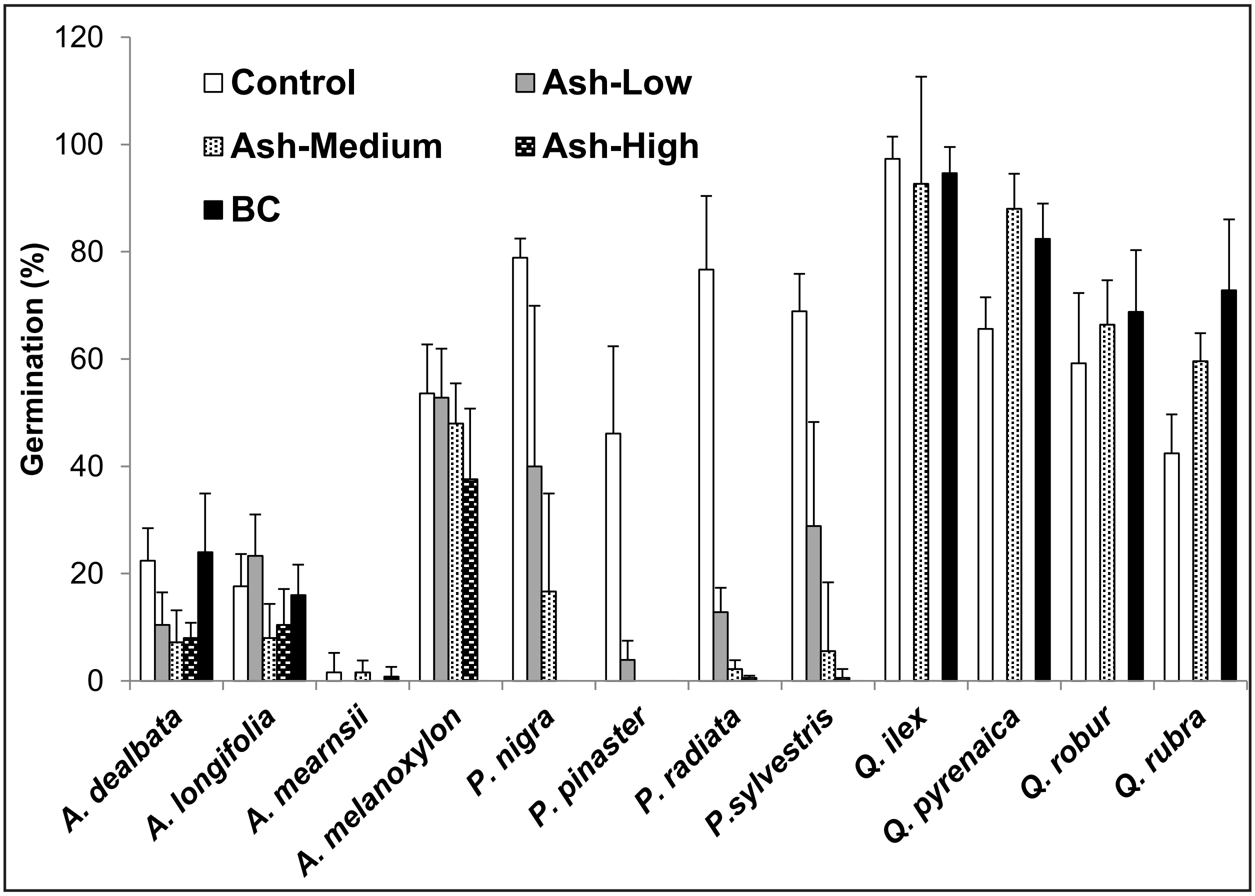

Figure 2. Mean percentage germination (±SD) reached by each species with each treatment. BC = black carbon.

and Ash-High treatments ($P < 0.05$) in *A. longifolia*. In the four species of pine, the ash treatment significantly inhibited ($P < 0.001$) germination and this effect was stronger with increasing ash concentration.

3) The only *Quercus* species that showed significant differences between treatments ($P < 0.005$) was *Q. rubra*. In *Q. rubra,* results were very different from those obtained with the other species, which may be related to the particular compositions of the *Ulex*-derived ash and BC: the treatments Ash-Medium and BC both stimulated germination, with BC increasing germination by 30%.

T_{50}

The period required to reach 50% germination varied significantly among species (Figure 3). Each genus has a particular T_{50} pattern, with *Pinus* being the fastest to germinate followed by *Acacia* and, lastly, *Quercus*. With the control treatment, rates varied from 5 days to 10 days in the *Pinus* species studied, to 22 days to 70 days in the *Acacia* species, to 87 days to 112 days in the *Quercus* species. The ANOVA applied to the T_{50} data detected highly significant differences between species ($P <$ 0.0001) and the interaction (species × treatment) was also significant ($P < 0.005$), which is indicative of differential responses to treatment for every species. Using the Duncan test, five groups of species were detected: (1)

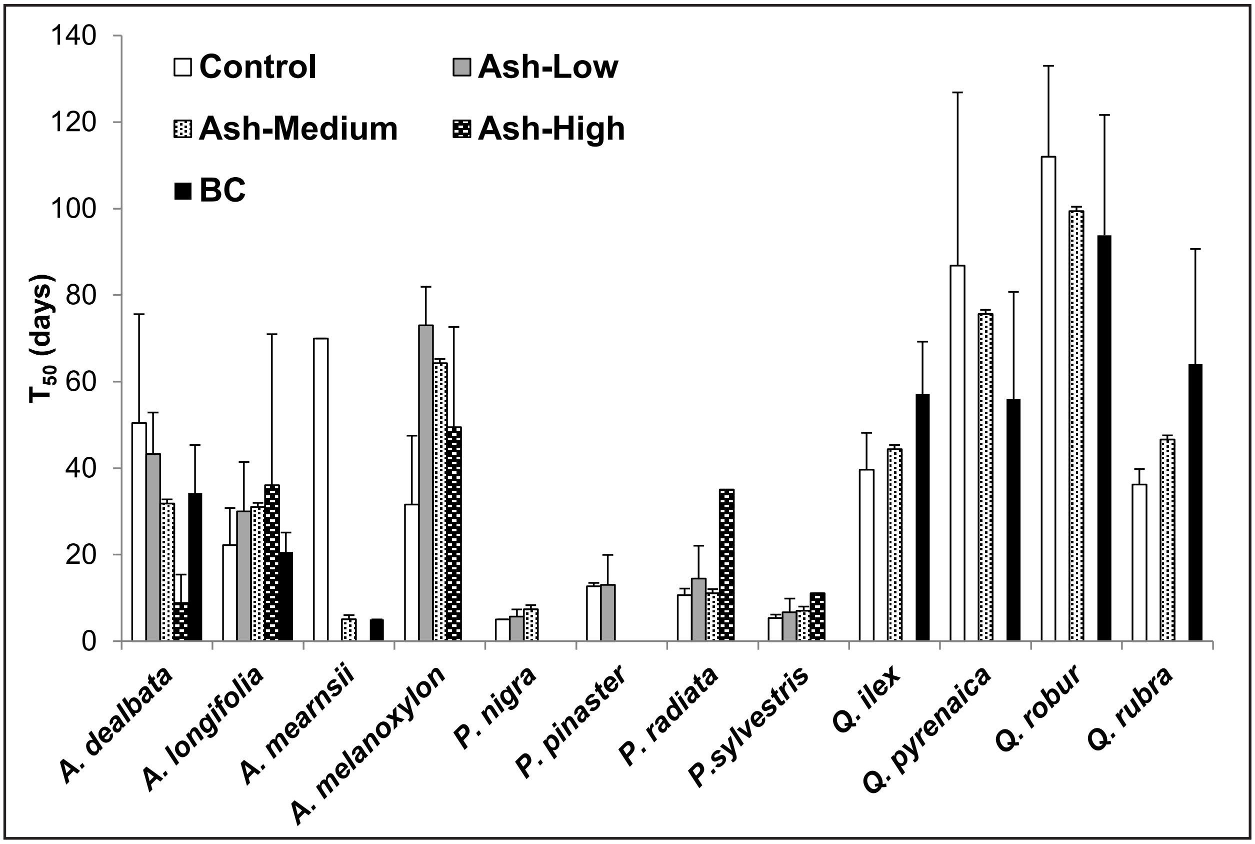

Figure 3. T_{50} (in days) and SD reached by each species with each treatment. BC = black carbon.

the four species of *Pinus* and *A. mearnsii* with the shortest average T_{50}; (2) *A. dealbata* and *A. longifolia* with T_{50} values between 23 days and 27 days; (3) *Q. ilex*, *Q. rubra,* and *A. melanoxylon* between 47 days and 55 days; (4) *Q. pyrenaica* with 73 days; and (5) *Q. robur* with an average T_{50} of 102 days. Analysing each species separately, we only found significant differences in treatments in the T_{50} of *A. dealbata* ($P < 0.001$), *A. melanoxylon* ($P < 0.005$), and *Q. ilex* ($P < 0.05$). The most striking result is that the Ash-Medium and Ash-High treatments significantly accelerate germination of *A. dealbata*. In *A. melanoxylon,* the Ash-Low and Ash-Medium treatments slow down germination, and in *Q. ilex*, BC produces that same effect. In the other species, no significant differences were detected between control and ash or BC treatments.

Temporal Distribution of Germination

The temporal distribution of germination was different for the species studied, even though there were certain similarities among species of the same genus (Figure 4). *Pinus* species took between 10 days and 20 days to complete their germination, *Acacia* about three months, *Q. rubra* and *Q. ilex* about 5 months, and *Q. pyrenaica* and *Q. robur* approximately one year. The responses to ash and BC treatments were also different. In the *Pinus* genus, during the first days, Control germination exceeded that of the treatments. For *Acacia*, the effects of the treatments on germination became visible only after 8 days and were less intense. For *Quercus*, germination was high and prolonged over time. The four *Quercus* species tested with Ash-Medium and BC treatments gave higher germination rates than the Control treatment during virtually the whole

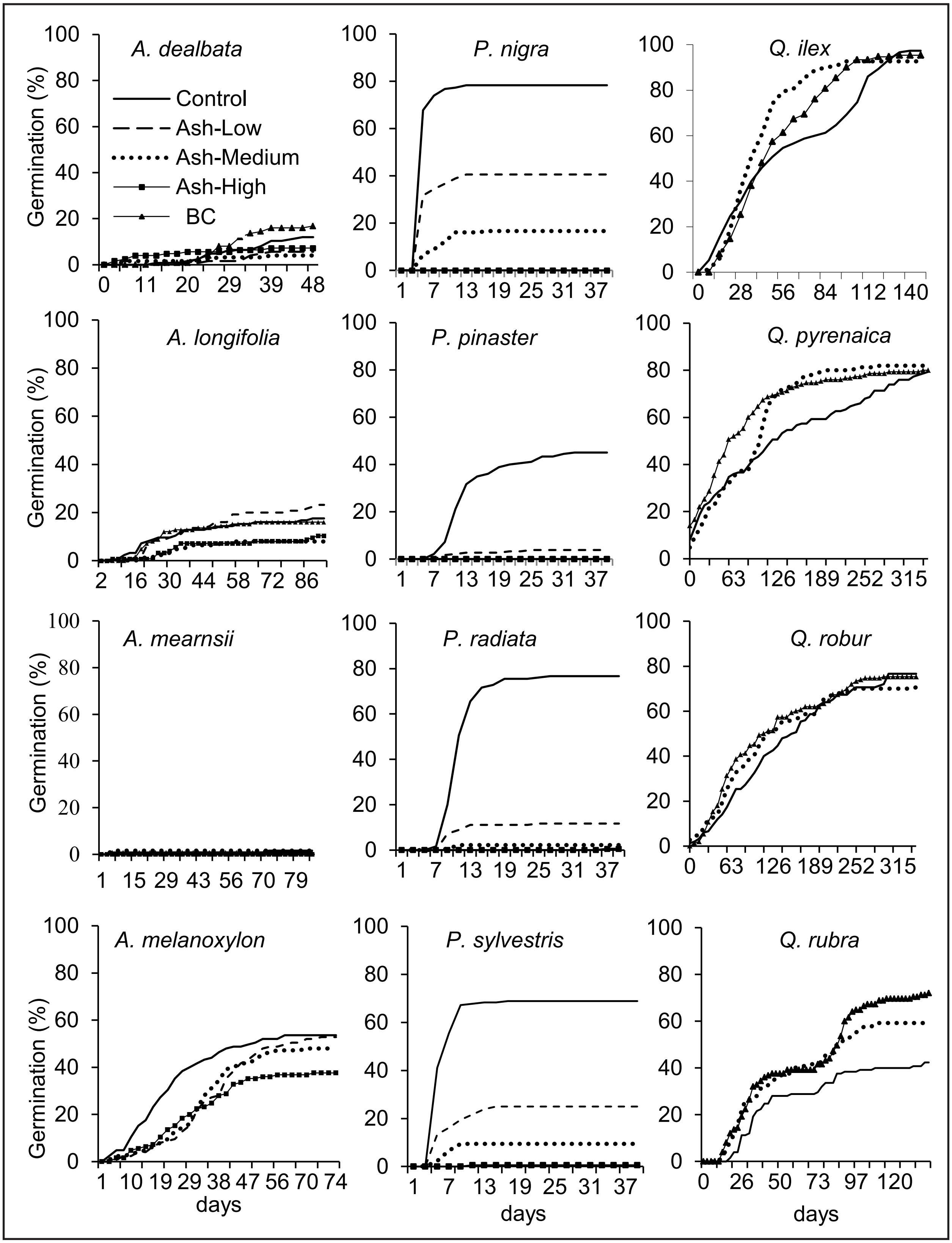

Figure 4. Temporal distribution of germination for each species and their treatments. BC = black carbon.

period, even though at the end the germination rates levelled out in all species except in *Q. rubra*. This last result is probably related to the different ash and BC used on *Q. rubra* and not an inherent difference between *Q. rubra* seeds behaviour with respect to the other *Quercus* species.

DISCUSSION

From the molecular properties of the BC samples, the results suggested that thermal impact was strongest for the *Quercus* BC, followed by *Pinus*, *Acacia,* and finally *Ulex*. All BC samples should be considered as strongly charred specimens, well past the phase of transition chars according to the scheme of Keiluweit *et al.* (2010). In comparison with laboratory-produced BC thermosequences under anaerobic conditions, they would have formed at an equivalent temperature of 450 °C to 600 °C (Kaal *et al.* 2012), which is well within the range generally considered suitable for biogeochemically "stable" or "recalcitrant" BC production (IBI 2012). The gorse BC sample produced large amounts of benzaldehyde, benzyl alcohol, and benzoic acid, which have not been observed previously in BC pyrolysates, at least not in such high proportions, and they were not abundant in anaerobically produced laboratory BC from *Ulex* wood (Kaal *et al.* 2012), suggesting that they are a result of the specific formation conditions such as condensation of leaf products.

Obviously, the high variability in the Control germination rates is related to the reproductive strategies acquired by each genus, developed through evolution. In *A. mearnsii*, *A. melanoxylon*, *Q. ilex*, *Q. pyrenaica,* and *Q. robur,* the different treatments had no significant effects on germination behaviour. The species of the *Quercus* and *Pinus* genera produce seeds with soft coats, while the seeds of *Acacia* have hard coats on which temperature shock has a special role in germination by physically breaking the dormancy (Rivas *et al.* 2006, Arán *et al.* 2013). The species with soft seed coats tend to show high Control germination rates as compared to species with hard seeds coats. Usually, germination of hard seeds is stimulated by heat or other agents that produce scarification (Ferrandis *et al*. 1999, Kimura and Islam 2012) and this explains the low germination percentages in *Acacia*. A lack of germination response to ash treatment has also been documented for species such as *Calluna vulgaris* (L.) Hull, *Erica umbellata* L., or *Salvia iodantha* Fernald. (González-Rabanal and Casal 1995, Zuloaga-Aguilar *et al.* 2011). In *A. dealbata, A. longifolia,* and the four *Pinus* species, the ash treatments usually inhibited germination and this effect increased with increasing ash concentration. These results are consistent with those of Trabaud and Casal 1989, Reyes and Casal 2004, and Kemball *et al.* 2010. According to González-Rabanal and Casal (1995), the decrease of germination percentages can be related to the sensitivity of seeds to the high osmotic pressure induced by the ash.

For *Q. rubra,* the results were very different as ash and BC both stimulated germination behaviour, which is probably a consequence of the particular chemical composition of the gorse-derived ash and BC applied to this species. Firstly, the ash of *U. europaeus*, which has N-fixation nodules in its root system, has a much higher N content (12 g kg^{-1}) than the 6 g kg^{-1} for pine species (Soto *et al.* 1997, Solla-Gullón *et al.* 2004). Light *et al.* (2005) and Downes *et al.* (2013) showed that N-rich ashes are stronger germination stimulating agents than N-poor analogues. For the gorse BC, on the other hand, the molecular properties showed that it had been subjected to the weakest thermal modification and produced remarkably high proportions of benzaldehyde, benzyl alcohol, and benzoic acid, probably of the same benzoic acid-based precursor. These substances may be responsible for inhibiting the development of fungi and bacteria (Tfouni and Toledo 2002, Drăcea *et al.* 2008). The seeds of *Q. rubra* germinate for almost five months and during this period they are often subjected to microbial attack from bacteria and fungi that would have been present on the seeds when they were picked in the field (even though they may also enter the system in the laboratory itself). Therefore, the particular nature of the gorse BC may have protected the *Q. rubra* seeds against this microbial attack, thereby enhancing its germination rate. On the contrary, the BC of the *Quercus* genus is devoid of benzaldehyde and benzyl alcohol, thereby not exerting such protection to the seeds of the other *Quercus* species studied. In the Mediterranean Basin, the number of species that are chemically stimulated by a fire agent (BC, ash, or smoke) is much smaller (Keeley and Baer-Keeley 1999, Reyes and Trabaud 2009), while physical stimulation (heat shock) is more common (Rivas *et al.* 2006, Moreira *et al.* 2010).

The T_{50} results are similar to those of other studies that found short germination rates in *Pinus* species (Neeman *et al.*1993; Reyes and Casal 1995, 2001; Escudero *et al.*1997; Álvarez *et al.* 2007), intermediate mean rates in *Acacia* and *Quercus* species (Li and Romane 1997, Valbuena and Tárrega 1998, Aref *et al.* 2011, Zuloaga-Aguilar *et al.* 2011, Arán *et al.* 2013), and very long rates in some *Quercus* species (Reyes and Casal 2006). Some ash treatments accelerate germination of *A. dealbata* significantly, whereas the most common response found was the non-modification of germination time (González-Rabanal and Casal 1995, Escudero *et al.* 1997, Reyes and Casal 1998).

In the *Pinus* genus, it is common to find short germination distribution periods as well (Escudero *et al.* 1997, Ganatsas and Tsakaldimi 2007). In the *Acacia* genus, germination distribution in response to treatments was delayed and less pronounced. Other studies with different species of the *Acacia* genus found both early (Aref *et al.* 2011) and medium germination (Zuloaga-Aguilar *et al.* 2011). In the *Quercus* genus, germination was high and prolonged over time, coinciding with the findings of Li and Romane (1997).

In a post-forest fire scenario, *Pinus* species would germinate quickly if sufficient water is available in the soil, but the inhibiting effect of ash on its germination will create few seedlings (Reyes *et al.* 2015). *Acacia* species initiate their germination rapidly and they maintain high germination rates over the first three months despite of the presence of ash or BC, and during this period the ash and BC may get substantially diluted or eliminated through runoff, thereby mitigating the inhibitive effect of ash and BC after these three months (Rumpel *et al*. 2009). The *Quercus* species, on the one hand, have such a long germination time that their seeds can take advantage of the time when environmental features are more advantageous for germination and, on the other hand, are less sensitive to physical and chemical conditions than other species. In *Q. rubra*, BC stimulates germination but it does not change its time distribution trend. The regeneration of plant populations after fire depends on many factors such as the wildfire season (Trabaud 1981), local climatic regimes, weather conditions following wildfire, and especially fire severity (Kemball *et al*. 2006). After severe fire events, the ash quantity on the soil may be very small and then the inhibitive effects of ash on germination will be reduced.

CONCLUSIONS

The twelve tree species studied show different responses to the fire agents (ash and BC), both in their germination percentages and their times. In the species studied of the *Acacia* and *Pinus* genera, ash and BC either do not modify germination, or they inhibit it. In three species of *Quercus,* none of the agents modify germination and, in turn, both stimulate it in *Q. rubra*. The *U. europaeus* BC sample has a particular molecular composition producing abundant benzoic acid and benzyl alcohol upon pyrolysis, contrary to the other BC samples analysed. Its stimulating effect on the germination of *Q. rubra* seeds may be related to these components.

More studies are needed to give further insight into the causes of the differential germination response of these species to ash and BC generated by forest fires. Even though this study was focused on twelve important forest ecosystem taxa, studies on the different effects of ash and BC in silvicultural and agricultural contexts involving BC amendments are urgently needed. Finally, the results suggest that multidisciplinary attempts are fundamental to unravel the complexity of the interactions between ash, BC, and seed germination behaviour in fire-affected areas.

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