

Recovering *Quercus* species on reclaimed coal wastes using native shrubs as restoration nurse plants



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ABSTRACT

Facilitative interactions among plants enable a species persist in stressful environments, but its use as a restoration technique of plant cover in degraded man-made ecosystems requires more accuracy. Here, a two-year field experiment was conducted to determine if native colonizer shrubs (*Genista florida* and *Cytisus scoparius*) used as nurse plants enhance *Quercus petraea* and *Quercus pyrenaica* reintroduction in reclaimed open-cast coal mines in Northern Spain. Of each tree species, 400 seedlings and 940 acorns were planted under four different treatments combining the influence of nurse shrubs and herbivory/predation upon *Quercus* seedlings and acorns. In each experiment, acorn emergence and seedling survival and growth were monitored. Shrubs enhanced seedling survival and growth and acorn emergence, in comparison with open spaces. Seedling survival was <20% in open areas and >90% under shrubs after the first summer. Acorn predation was a limitation, but not seedling herbivory. Seedling survival and growth were greater for *Q. pyrenaica* than *Q. petraea*, while *Q. petraea* showed better acorn emergence and survival of emerged acorns. The use of shrubs as nurse plants to improve environmental conditions is promising for the effective reintroduction of late successional species in reclaimed mined sites. The best results are obtained through *Q. pyrenaica* seedlings under shrubs. This avoids problems such as acorn predation and germination failure. The ontogenetic stage and the stress tolerance of the target species should be taken in consideration in order to increase the effectiveness of this restoration technique.

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1. Introduction

Facilitation in plant communities encompasses non-trophic, beneficial interactions between organisms (Brooker et al., 2008). In the last years, facilitation is achieving its own field of research, due to its contribution to our understanding of ecological systems, ecosystem restoration and its potential evolutionary impacts among other topics (Brooker and Callaway, 2009). However, there are still a lot of uncertainties about how facilitative interactions work (Brooker et al., 2008), especially in novel ecosystems created due to restoration of man-made impacts (Hobbs et al., 2009).

The plant–plant interaction outcome is a complex process which depends on many factors, such as species identity,

environment and the life stage of the interacting organisms among others (Gómez-Aparicio et al., 2004; Prévosto et al., 2012). Conceptual models explaining facilitation (see Bertness and Callaway (1994) for the stress-gradient hypothesis, and Maestre and Cortina (2004) or Michalet et al. (2006) for the hump-shaped hypothesis) do not still represent the reality of the processes properly (Brooker et al., 2008; López et al., 2013). Although it is still not clear when neighbour effects will be positive, they are more probable in limiting habitats such as drylands (Brooker et al., 2008), or when the site is further from the species niche optimum (Gross et al., 2010; Liancourt et al., 2005), like in new-ecosystems such as reclaimed mines. In fact, facilitation processes enable the creation of new niche spaces in stressful environments for species establishment (Bruno et al., 2003).

Recent studies have suggested that facilitation processes can be a tool to enhance plant restoration in disturbed environments, where harsh environmental conditions or herbivory are major constraints (Brooker et al., 2008). The usage of shrubs as restoration tools under the Mediterranean conditions of the

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Iberian Peninsula is well-known (e.g. Castro et al., 2002; Gómez-Aparicio et al., 2004, 2008). Indeed, this has been the topic of two meta-analyses and a review already (Gómez-Aparicio, 2009; Padilla and Pugnaire, 2006; Verdú et al., 2012). However, although some progress have also been achieved by testing facilitation processes as restoration technique in traditional anthropogenic systems, such as grasslands or croplands (Cuesta et al., 2010; Pueyo et al., 2009; Soliveres et al., 2013), more knowledge is needed in more drastically man-disturbed ecosystems, such as open-cast mines. An efficient use of facilitation in restoration projects requires more accuracy about the results of particular target and nurse species interactions under specific problematic conditions. Thus, widening the range of species and environments considered in the facilitative restoration experiments seems necessary.

Coal mining is an extended activity in Northwest Spain (Alday et al., 2011a), where *Quercus petraea* at its southernmost distribution limit and *Quercus pyrenaica* almost endemic of Iberian Peninsula (do Amaral Franco, 1990) are major forest species. The restoration of these native forest species in mined sites is very important for the provision and maintenance of ecological functions, biodiversity and landscape values (Alday et al., 2011b). In recent times, the most common mine restoration approach within this region has been to reshape the mined landform, improve the baseline soil-forming materials, and then introduce herbaceous seeds by hydroseeding (González-Alday et al., 2008). However, the recovery in these mine sites of late-successional broadleaf species present before the mining operations, such as *Q. petraea* and *Q. pyrenaica*, is a hard task (Alday et al., 2014). It is well known that in Mediterranean ecosystems, forest species regeneration suffers from two major constrains: (i) summer drought (Pugnaire et al., 2011), being increased in reclaimed mined sites by a lack of soil structure such as in forest systems (Alday et al., 2012), and (ii) herbivory, which can occur through trampling and browsing by livestock and wild ungulates, but also by means of acorn predation for *Quercus* species (see Gómez et al., 2003; Smit et al., 2008). In order to overcome these two constraints, facilitation mediated by shrubs has been proposed for the reforestation of Mediterranean systems (Castro et al., 2006; Gómez-Aparicio et al., 2004; Rey et al., 2009), which could also be implemented for mining sites (see Alday et al., 2014). Facilitation mediated by shrubs can help to reduce summer drought stress (Smit et al., 2008), improving soil properties (Pugnaire et al., 2004), and providing protection against herbivory (Smit et al., 2006). Thus, in the present study we assessed the influence of nurse shrubs during two growing seasons on reforested seedlings and acorns of two *Quercus* species (*Q. petraea* and *Q. pyrenaica*) in a reclaimed open-cast coal mine. The aim of the experiment was to identify the effectiveness of using native colonizer shrubs as nurse plants to reintroduce the two main tree species present before the mining operations. It must be considered that in these mined sites the summer drought effects are increased by the lack of developed soil, restricting the development of *Quercus* species. Moreover, herbivory usually reduces the establishment success of these species. Therefore, the use of shrubs as nurse plants might help to decrease these constrains. Here, our hypotheses were: (1) the effect of shrubs and enclosures would be positive for the survival of the two *Quercus* species seedlings; (2) shrubs and enclosures would also be beneficial for seedling growth; (3) acorn emergence and subsequent seedling survival of the two *Quercus* species would be positively affected by shrubs and mesh protection in the mine as by mesh protection in the forest microhabitat; and (4) different patterns between species for the studied parameters would expect to be found according to their different ecological requirements.

2. Materials and methods

2.1. Site description and mine restoration treatment

The experiment was located in a 7 ha reclaimed open-cast coal mine near Guardo, Palencia, Northern Spain (latitude 42°48'N, longitude 4°52'W, ca. 1200 m a.s.l.). The climate is sub-humid Mediterranean (Alday et al., 2011a), with an annual mean temperature of 9.3 °C and average annual precipitation of 977 mm (1971–2007 temperature means and 1932–2007 precipitation means from Guardo meteorological station). Rainfall is not distributed regularly throughout the year, with a pronounced dry season in summer (July and August). In addition, precipitations of the study years were rather different. Precipitation from September 2010 to June 2011 reached 778.2 mm (80.2 mm in July–August), whereas only 567.8 mm were registered from September 2011 to June 2012 (26.5 mm in July–August). The vegetation surrounding the mine consisted of broad-leaved woodlands dominated by *Q. pyrenaica* and *Q. petraea*, and some shrubs such as *Cytisus scoparius* and *Genista florida*.

The open-cast mine was reclaimed in October 2000, using a combination of topsoil addition with a very poor seed bank (González-Alday et al., 2009), followed by hydroseeding with a grassland species mixture (for further reclamation details see Alday et al., 2011a). The reclaimed area had a patchy natural colonization of shrubs, mainly *C. scoparius* and *G. florida*, being grazed freely by animals (deer, wild boar, cattle and horses). The current soil texture is described as clay loam with a pH of 6.5 and with an effective depth of 10–15 cm (López-Marcos, 2012).

2.2. Experimental design and data collection

2.2.1. Seedlings experiment

The experimental set-up in the mine area consisted of four main-plots (30 m × 30 m, flat area): two fenced main-plots and two no-fenced main-plots with shrub/open subplots into each one, to assess the combined influence of nurse shrubs and grazing upon *Quercus* seedlings. The treatments were: (a) no-grazing and no-shrubs (fenced open areas with no shrub cover, OF); (b) no-grazing and shrub cover (fenced areas under shrubs, SF); (c) grazing and no-shrubs (no-fenced open areas, ON); (d) grazing and shrub cover (no-fenced areas under shrubs, SN). The species used as nurse shrubs were two natural mine sites colonizers, i.e. *C. scoparius* and *G. florida*, with similar vertical structure (mean height 222 ± 6.6 cm). Both species are non-thorny leguminous shrubs, 2–2.5 m tall, capable of actively fixing the atmospheric nitrogen (Talavera et al., 1999). At the same time, both species are considered into the same functional group sharing common characteristics (i.e. structure and leaf phenology), and they usually co-exist in the mined areas where they regenerate very mixed. As a consequence and based on the methodology carried out in studies using similar functional group species (Gómez-Aparicio et al., 2004), they were not differentiated in the experiment. The enclosures consisted of wire mesh (2 m high, mesh hole: 5 cm width × 15 cm length) fixed through poles and were constructed to prevent ungulates and rabbits grazing the sub-plots.

Within each main-plot, ten sub-plots (five under shrubs and five in open areas) were allocated randomly ca. 4 m apart from each other (each sub-plot ca. 2 m × 2 m; when sub-plots were under shrubs they gathered 2–3 shrubs). For example, in a fenced main-plot five OF sub-plots and five SF sub-plots were located, whereas in a non-fenced main plot five ON sub-plots and five SN sub-plots were set. In each sub-plot 10 tagged seedlings (one-year-old) of each species (400 seedlings of *Q. petraea* and *Q. pyrenaica* in total) were planted in March 2011. Seedlings (Cordillera Cantábrica Provenance), grown in cylindrical pots (type: S.L 35, 235 cm³, 16 cm

deep \times 5 cm wide) filled with peat, were provided by the central nursery of Junta de Castilla y León. They were placed separated from each other by 30 cm, in two rows when they were planted in open areas and in one row around the shrub stem, 20 cm far from it, when they were planted under shrubs (see Fig. A1 in Appendix A). When the lowest branches of the shrub prevented the seedlings to be placed at that distance, seedlings were planted as near to the stem as possible (ca. 30 cm). Planting was carried out using a small auger (20 cm \times 6 cm) to minimize the damage to the shrub roots and soil structure disturbance. In all the cases, the soil was returned to the holes and firmed around the root collar of the seedlings. One month after plantation, post-plantation survival success was checked through seedling survival evaluation, considering them as dead when stems were dry and no chlorophyll was visible (99.8% of seedlings survived).

After this first survival evaluation, seedlings were monitored during two consecutive years (2011–2012). In 2011 the survey was more exhaustive in order to control any post-plantation problem. We recorded: (1) survival in spring, summer and autumn 2011 and summer and autumn 2012; and (2) leader shoot growth in autumn 2011 and 2012. Survival was corrected through time for those seedlings that were apparently dead and resprouted in the next surveys.

2.2.2. Acorn experiment

In November 2011 we collected *Q. petraea* and *Q. pyrenaica* acorns from nearby forests and a sowing experiment was performed with the same experimental set-up than for seedling experiment. In each sub-plot (1 m \times 0.25 m), 20 acorns of each species were buried horizontally into the soil at 2–3 cm (40 acorns per sub-plot, two per hole and holes systematically separated from each other by 7–8 cm), protecting the surface against rodents with a labelled wire mesh (1.2 cm mesh width) fixed by nails (see González-Rodríguez et al., 2012). In addition, to assess the acorn performance in the natural forest, we also set up 10 sub-plots of the same characteristics protected by mesh in the nearby forest (see Fig. A2 in Appendix A). Non-viable acorns (empty or preyed upon by insects) were excluded by floating in water (Gómez et al., 2003).

However, in March 2012, some sub-plots were altered by animals (livestock and wild ungulates) and humans; some wire meshes disappeared and in a few sub-plots the soil surface was dug by wild boars. Therefore, we excluded destroyed sub-plots changing the experimental design so that we could analyse the effect of environmental conditions and protection against rodents (final number of sub-plots and acorns: 47 sub-plots, 940 acorns per species). Thus, the treatments considered were: in the mine (1) under shrubs with mesh (SM; $n = 17$), (2) open areas with mesh (OM, $n = 7$), (3) under shrubs without mesh (SN, $n = 5$), (4) open areas without mesh (ON, $n = 13$); and (5) in the forest with mesh (FM, $n = 5$). For each acorn we recorded emergence and survival in March, May June, early and late July and October 2012.

2.3. Data analysis

All statistical analyses were implemented in the R software environment (2.15.2; R Development Core Team, 2012). Seedling survival and acorn emergence and survival were analysed using lme4 package for generalised linear mixed models (GLMM; Bates et al., 2013) and growth analysis was carried out with nlme package for linear mixed models (LMM; Pinheiro et al., 2013).

First, the roles that shrubs, fenced areas, planted species and time played in seedling survival was analysed, using GLMMs. In model construction we started with the full model including the interaction between presence/absence of shrubs and fence, species and time as fixed effects. In these model, random effects accounting for spatial and temporal pseudoreplication were

included (main-plot, sub-plot and time; Pinheiro and Bates, 2000). Second, GLMMs were used to analyse the effect of shrub, mesh, site (mine or forest), species and time on acorn emergence and survival (fixed effects), whereas time and the hierarchical structure of the design was included as random (main-plot/sub-plot).

The survival and emergence data showed a binary response (live or not); therefore in both analyses a binomial error distribution with a logit-link function was used. Models were fitted using the adaptive Gaussian Hermite approximation and with a nAGQ = 5 (Bates et al., 2013).

Third, the analysis of growth considered only the seedlings which were alive at the end of the second growing season. The effect of shrub, fence, species and time was tested by using LMM, with normal distribution, where species, year and presence/absence of shrubs and fence were included as fixed factors and main-plot, sub-plot, individual and year as random factors. In this model, the homoscedasticity was corrected using the Varident function and defining different variances for each main-plot at each time (Pinheiro and Bates, 2000). In all analyses, model simplification guidelines followed Crawley (2007) using the Akaike information criterion (AIC; Pinheiro and Bates, 2000). Starting from the full model, the minimal adequate GLMM was obtained by sequential removal of non-significant model terms until no further reduction in AIC was observed.

3. Results

3.1. Seedling survival

The GLMM showed that there was a significant shrub \times time \times species interaction (Table 1a). However, fenced areas (SF, OF) had no significant effect on seedlings survival. Seedling survival descended significantly through time for both the species, being *Q. pyrenaica* survival always greater than *Q. petraea* survival (Fig. 1), and for both species being greater under shrubs than in open areas. The seedling mortality in open areas occurred mostly at the beginning of the first dry season (June and July 2011, 3rd and 4th month after plantation; Fig. 1), whereas mortality under shrubs occurred more gradually along the two years (e.g. highest mortality increment under shrubs between October 2011 and July 2012, 6th and 16th month after plantation: *Q. petraea* = 39.7%, *Q. pyrenaica* = 30.6%, Fig. 1). Thus, in the first autumn (October 2011, 6th month after plantation), survival under shrubs was greater than in open areas (*Q. petraea* = 90.7%, *Q. pyrenaica* = 91.0% vs. *Q. petraea* = 5.5%, *Q. pyrenaica* = 17.0%), although after the second dry season (July and

Table 1
Model parameters estimates derived from GLMM models for (a) seedling survival (Z-values), and (b) seedling growth (t-values). O-Pt: *Q. petraea* in open areas.

Fixed effects	Estimate \pm S.E.	Value	P
(a) Seedling survival			
Intercept_O-Pt	5.39 \pm 0.60	9.03	<0.001
Shrub	0.12 \pm 0.80	0.15	0.880
Time	-1.40 \pm 0.26	-5.40	<0.001
<i>Q. pyrenaica</i>	0.24 \pm 0.26	0.97	0.330
Shrub \times time	1.33 \pm 0.27	4.87	<0.001
Shrub \times <i>Q. pyrenaica</i>	0.45 \pm 0.50	0.91	0.364
Time \times <i>Q. pyrenaica</i>	0.16 \pm 0.04	4.45	<0.001
Shrub \times time \times <i>Q. pyrenaica</i>	-0.17 \pm 0.04	-3.72	<0.001
(b) Seedling growth			
Intercept_O-Pt	2.14 \pm 1.01	2.11	0.036
Shrub	5.69 \pm 1.03	5.52	<0.001
Time	-0.65 \pm 0.10	-0.65	0.519
<i>Q. pyrenaica</i>	1.30 \pm 0.35	3.72	<0.001
Shrub \times time	-4.06 \pm 1.06	-3.82	<0.001

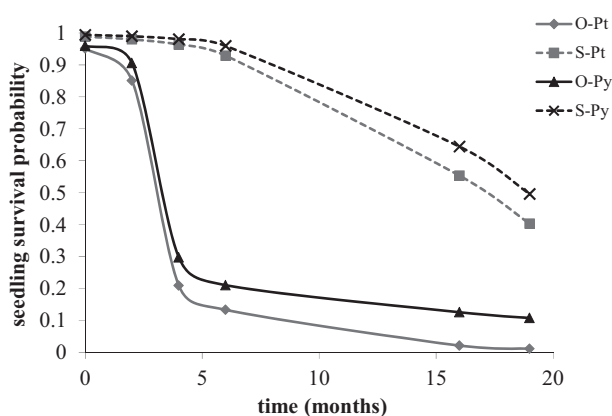


Fig. 1. Seedling survival for *Q. petraea* and *Q. pyrenaica* species in open areas and under shrubs through the two growing seasons. The fitted line represents the minimal adequate GLMM. O-Pt: *Q. petraea* in open areas, S-Pt: *Q. petraea* under shrubs, O-Py: *Q. pyrenaica* in open areas, S-Py: *Q. pyrenaica* under shrubs.

August 2012, 16th and 17th month after plantation), the seedling survival had decreased in both areas (under shrubs: *Q. petraea* = 44.6%, *Q. pyrenaica* = 54.3%; in open areas: *Q. petraea* = 2.5%, *Q. pyrenaica* = 11.5%).

3.2. Seedling growth

Growth performance was analysed only in 27.8% of the seedlings, i.e. those alive at the end of the second growing season (October 2012). The LMM showed that there was a time × shrub interaction (Table 1b, Fig. 2). Thus, the first year seedlings of both the species under shrubs grew more than those in open areas (shrub × time: $F_{1,220} = 14.57, P < 0.001$). On the other hand, seedling growth was stable through years in open areas (first year: 2.7 ± 1.0 cm vs. second year: 2.6 ± 1.3 cm for *Q. petraea*; first year: 3.2 ± 0.7 cm vs. second year: 2.5 ± 0.5 cm for *Q. pyrenaica*; mean ± S.E.; Fig. 2), whereas the growth of seedlings under shrubs was reduced significantly in the second year regarding the previous year (first year: 8.3 ± 0.5 cm vs. second year: 2.9 ± 0.3 cm for *Q. petraea*; first year: 8.9 ± 0.5 cm vs. second year: 4.7 ± 0.3 cm for *Q. pyrenaica*; Fig. 2). It is noteworthy to mention that the species had a significant effect on growth, since *Q. pyrenaica* seedlings grew

Table 2

Model parameters estimates derived from GLMM models for acorn emergence (a) in mine areas (ON-Pt: *Q. petraea* in open areas without mesh), and (b) in mine areas with mesh and the forest (FM-Pt: *Q. petraea* in forest areas with mesh).

Fixed effects	Estimate ± S.E.	Z-value	P
(a) In mine areas			
Intercept_ON-Pt	-7.03 ± 0.41	-16.96	<0.001
Mesh	2.58 ± 0.45	5.80	<0.001
Shrub	-0.58 ± 0.32	-1.82	0.068
<i>Q. pyrenaica</i>	-0.67 ± 0.15	-4.47	<0.001
Time	0.08 ± 0.01	9.55	<0.001
Shrub × <i>Q. pyrenaica</i>	-0.53 ± 0.19	-2.88	0.004
(b) In mine areas with mesh and forest			
Intercept_FM-Pt	-4.94 ± 0.38	-12.96	<0.001
Mine_mesh	-0.26 ± 0.39	-0.68	0.498
<i>Q. pyrenaica</i>	-1.71 ± 0.41	-4.13	<0.001
Time	0.10 ± 0.01	11.15	<0.001
Mine_mesh × <i>Q. pyrenaica</i>	1.32 ± 0.27	4.86	<0.001
<i>Q. pyrenaica</i> × time	-0.02 ± 0.01	-2.06	0.039

more than those of *Q. petraea* ($F_{1,196} = 13.87, P < 0.001$; Table 1b and Fig. 2).

3.3. Acorn emergence and survival

Acorn emergence started in June 2012 (29 weeks after seeding) and in October 2012 there were still new emerged acorns. In the mine, both species showed similar trends of emergence within each treatment (*Q. petraea*: SM = $40.6 \pm 5.7\%$, OM = $21.4 \pm 6.6\%$, ON = $4.6 \pm 2.4\%$, SN = 0%; *Q. pyrenaica*: SM = $21.2 \pm 4.0\%$, OM = $12.9 \pm 4.2\%$, ON = $3.5 \pm 1.6\%$, SN = $1.0 \pm 1.0\%$; mean ± S.E). However, GLMM showed two interesting patterns (Table 2a). First, there was a significant positive effect of mesh on emergence independently of shrub cover or the *Quercus* species considered. In both cases *Quercus* species had more probability of emerging under mesh sites (Fig. 3a). Second, there was a shrub × species interaction, being *Q. petraea* acorns emergence significantly greater under shrubs than in open areas (Fig. 3b). Moreover, *Q. petraea* had always greater emergence probability than *Q. pyrenaica*, which showed slightly greater emergence values under shrubs. Comparing mine areas with mesh against forest areas (Table 2b), GLMM results indicated that the emergence probability rose with time for both species, showing *Q. petraea* a greater and

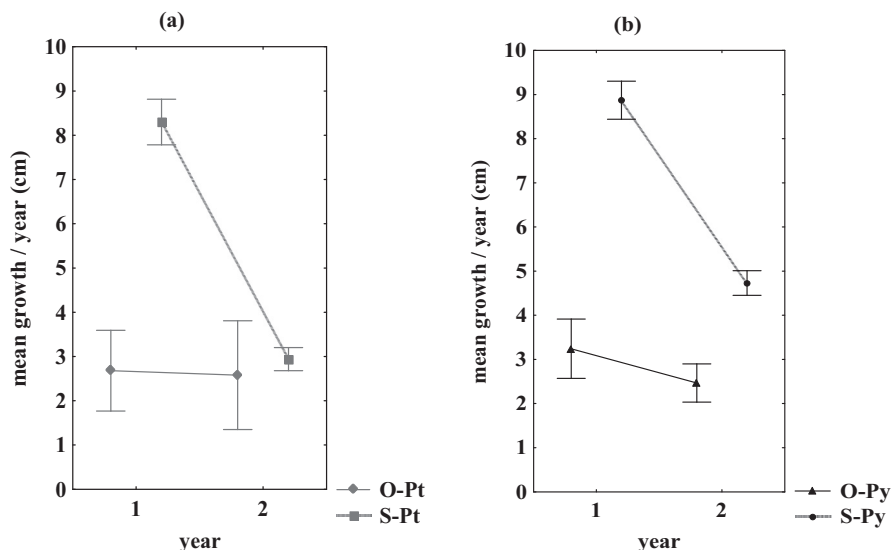


Fig. 2. Seedling mean growth of (a) *Q. petraea* and (b) *Q. pyrenaica* species in open areas and under shrubs at the end of each growing season. Bars represent the standard error. O-Pt: *Q. petraea* in open areas, S-Pt: *Q. petraea* under shrubs, O-Py: *Q. pyrenaica* in open areas, S-Py: *Q. pyrenaica* under shrubs.

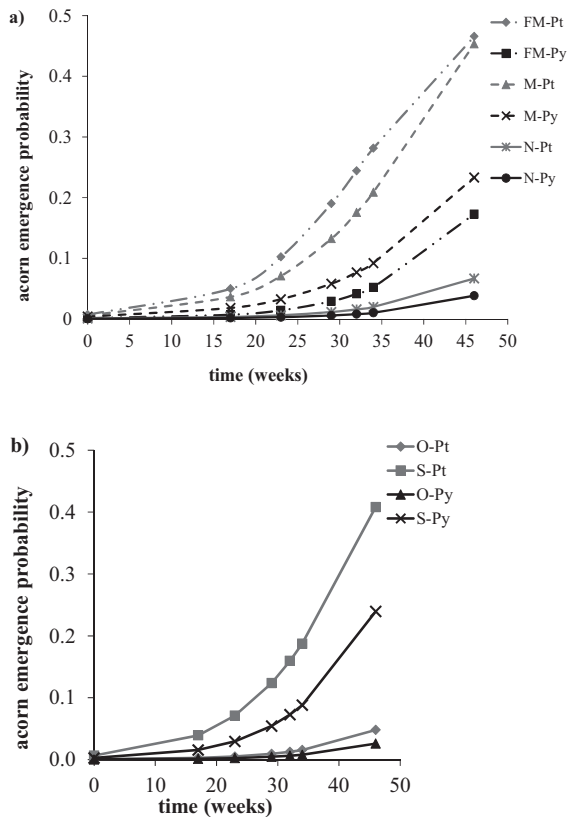


Fig. 3. (a) Probability of acorn emergence of *Q. petraea* and *Q. pyrenaica* species in the forest with mesh and in the mine areas with mesh and without mesh through the growing season. FM-Pt: *Q. petraea* in the forest with mesh, FM-Py: *Q. pyrenaica* in the forest with mesh, M-Pt: *Q. petraea* in mine areas with mesh, N-Pt: *Q. petraea* in mine areas without mesh, M-Py: *Q. pyrenaica* in mine areas with mesh, N-Py: *Q. pyrenaica* in mine areas without mesh. (b) Probability of acorn emergence of *Q. petraea* and *Q. pyrenaica* species in open areas and under shrubs in the mine through the growing season. S-Pt: *Q. petraea* under shrub, O-Pt: *Q. petraea* in open areas, S-Py: *Q. pyrenaica* under shrub, O-Py: *Q. pyrenaica* in open areas. For both (a) and (b), the fitted line represents the minimal adequate GLMM.

faster increase than *Q. pyrenaica* (Fig. 3a). Nevertheless, the difference in emergence among species was slightly lower in mine sites compared with forest areas.

In October 2012, the number of live seedlings from the sown seeds in the mine site (840 acorns per species) was very low and significantly higher in *Q. petraea* than in *Q. pyrenaica* (*Q. petraea* = 11.40%, *Q. pyrenaica* = 7.45%; Z -value = 3.96, $P < 0.001$), as in the forest (*Q. petraea* = 22.0%, *Q. pyrenaica* = 11.0%; Z -value = 2.28, $P = 0.023$). Within each treatment the survival percentages in the mine were for *Q. petraea*: SM = $24.1 \pm 4.9\%$, OM = $2.1 \pm 1.0\%$, ON = 0%, SN = 0%, and for *Q. pyrenaica*: SM = $15.6 \pm 3.7\%$, OM = $0.7 \pm 0.7\%$, ON = $1.9 \pm 1.6\%$, SN = 0%. In the mine, the survival of emerged seedlings was influenced by mesh, time, and species (Table 3a, Fig. 4). The survival was greater in areas covered with mesh (sub-plots with mesh: $14.5 \pm 2.7\%$, sub-plots without mesh: $0.7 \pm 0.6\%$; mean \pm S.E.), increasing significantly with time, and being greater in *Q. petraea* than in *Q. pyrenaica* in mesh sub-plots (*Q. petraea* with mesh: $17.7 \pm 4.0\%$, *Q. pyrenaica* with mesh: $11.3 \pm 2.9\%$). Moreover, at the end of the growing season there was no significant difference in species survival between forest microhabitat and the treatment SM (Table 3b), although *Q. petraea* survival was greater than *Q. pyrenaica* survival (Table 3b). In any case, the survival in no-shrub mine treatments (i.e. OM and ON) was lower compared with the forest treatment FM. SN has not been included in the analysis because no acorn survived.

Table 3

Model parameters estimates derived from GLMM models for (a) acorn survival in mine areas through the time (ON-Pt: *Q. petraea* in open areas without mesh), and for (b) acorn survival among mine areas and forest at the end of the growing season (FM-Pt: *Q. petraea* in forest with mesh).

Fixed effects	Estimate \pm S.E.	Z-value	P
(a) Through the time			
Intercept_ON-Pt	-8.55 ± 0.50	-16.95	<0.001
Mesh	3.13 ± 0.54	5.77	<0.001
<i>Q. pyrenaica</i>	-0.95 ± 0.10	-9.76	<0.001
Time	0.08 ± 0.01	12.05	<0.001
Shrub	0.77 ± 0.50	1.55	0.121
(b) At the end of the growing season			
Intercept_FM-Pt	-1.84 ± 0.51	-3.59	<0.001
ON	-3.15 ± 0.80	-3.93	<0.001
OM	-2.53 ± 0.84	-2.98	0.003
SM	0.53 ± 0.57	0.93	0.351
<i>Q. pyrenaica</i>	-0.57 ± 0.18	-3.20	0.001

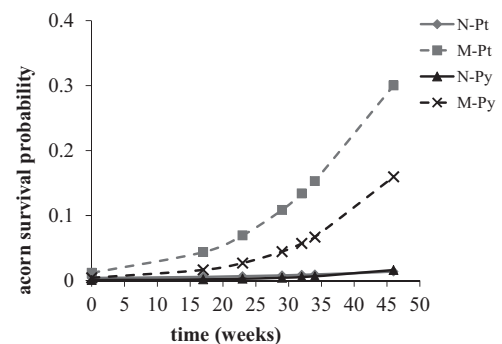


Fig. 4. Probability of acorn survival of *Q. petraea* and *Q. pyrenaica* species in mine areas with mesh and without mesh through the growing season. The fitted line represents the minimal adequate GLMM. M-Pt: *Q. petraea* in areas with mesh, N-Pt: *Q. petraea* in areas without mesh, M-Py: *Q. pyrenaica* in areas with mesh, N-Py: *Q. pyrenaica* in areas without mesh.

4. Discussion

4.1. Seedling survival

Shrubs enhanced seedling survival in comparison with seedlings planted in open areas. It seems that a positive effect on seedling survival exists when seedlings are set beneath shrub canopies in this mined site. The positive effects of shrubs on tree species in our experiment are in agreement with the general trend of neighbour effects among woody species, especially when the neighbour species are shrubs and the target species are trees (Gómez-Aparicio, 2009). This result supports the idea of using shrubs as ecosystem engineers in order to improve late-successional species establishment in mined sites (Alday et al., 2014; Bradshaw, 1997). The effect of shrubs in microclimate amelioration (Gomez-Aparicio et al., 2005) and the improvement of soil properties (e.g. fertilizing, water holding capacity; Pugnaire et al., 2004), as a whole, may help to reduce the hydric stress in seedlings under shrubs compared with seedlings in open areas, favouring their survival. Unfortunately, this experiment cannot identify the importance of each of these factors independently.

As expected on Mediterranean systems, the first summer after plantation is a critical period for seedling survival (Castro et al., 2006); here 48.6% of seedlings died in the first summer (May–September, 2011). However, the most affected individuals were those located in open areas, whereas most of the seedlings beneath shrub canopies survived after that period (88.8% died in open areas vs. 9.2% died under shrubs). These results pointed out the positive

effect of shrubs on seedling survival when conditions became worse. Alternatively, seedlings mortality under shrubs increased during the second year after plantation, probably because of the lower precipitations during the second growing season (September 2011–June 2012) that might have reduced the soil water availability in the mine. This dry period generated very hard conditions for seedlings survival in the mine, especially considering that water holding capacity of mine soil is clearly lower than in the nearby natural forest soil (1–3.5 g/cm² vs. 19.8 g/cm²; López-Marcos, 2012). Then, it seems that amelioration of environmental conditions by shrubs was not enough to satisfy the water requirements of studied seedlings during most stressful periods. Thus, although seedlings survive better beneath the shrub canopies, under more extreme dry seasons shrub cover is not enough to prevent the seedlings death. Similar studies have documented that extreme environmental severity prevent a significant effect of facilitation (Brooker et al., 2008), whereas studies in arid and semi-arid environments have also found a shift from positive to negative outcome between plants when water resources decrease, possibly because the facilitator cannot compensate for its own resource competition or rain interception (Maestre and Cortina, 2004).

Contrary to our expectations and findings in other areas of Spain (Gómez et al., 2003), enclosures did not enhance *Quercus* seedling survival suggesting, in principle, that ungulate browsing is not a major problem for *Quercus* regeneration in the area. Although *Quercus* species are not very attractive for some herbivores, like cattle, due to their high tannin concentrations (Rodríguez-Doce, 2010), ungulates could totally destroy seedlings by trampling or pulling up without consumption affecting their survival. However, in no-fenced areas shrubs as a protective mechanical barrier, even in the case of non-spiny plants (Gómez et al., 2001), could prevent seedling damages by ungulates and might hide the enclosures effect on *Quercus* seedling survival.

The different survival patterns of both species can be due to their differences in ecophysiological traits and responses to environment. *Q. petraea* is adapted to environments where resources are abundant, but it endures bad stressful conditions such as high radiation, water deficit or above-ground disturbances, whereas *Q. pyrenaica* presents traits related to a stress tolerance strategy such as a higher self-shading, root to shoot ratio, sprouting habit, and a more conservative growth strategy than *Q. petraea* (Rodríguez-Calcerrada et al., 2008). Those differences may explain why in our experiment *Q. pyrenaica* has shown higher survival throughout time compared with *Q. petraea*, like it happens in other drought-prone habitats (Rodríguez-Calcerrada et al., 2010). Therefore, it seems necessary to consider ecological requirements and characteristics of candidate species in order to select the most adapted species to the main site constrains before using shrubs as nurse plants. This species selection will determine the success of tree reintroduction and also their self-maintenance, as in the future those species also will influence the nutritional and microbial properties of the mine soil (Mukhopadhyay et al., 2013).

4.2. Seedling growth

It was hypothesized that there is a beneficial effect of shrubs and enclosures on seedling growth; however, only shrubs had a positive effect on growth, while enclosures did not affect it. The higher seedling growth found beneath shrub canopies in comparison with open areas is probably caused by less stressful light conditions, and better soil properties and water status under shrubs (López-Marcos, 2012). These results contrast with other studies (Gómez-Aparicio et al., 2005; Marañón et al., 2004), where despite finding higher seedling survival in shady habitats, under neighbour plants the seedling growth decreased or did not

improved compared with open areas. Neutral or negative interactions among nurse species and target species are usually found in facilitation studies when growth is considered, in contrast to the general positive effects upon survival (Gómez-Aparicio, 2009), but sometimes stem elongation can be higher in shaded microsites than in open areas (Pérez-Ramos et al., 2010). In addition, although *Quercus* seedlings under shade conditions may grow less than those under full light, the shortage of water may also reduce their growth, so the equilibrium between both factors determines the final response (Baraza et al., 2004; Marañón et al., 2004). In our study conditions, restoration with nurse shrubs does not confront survival against growth, but favours both, resulting in a promising technique for *Quercus* reintroduction in mine sites.

During the second year after plantation seedling growth beneath shrub canopies decreased in comparison with the first year, and this was followed by differences in growth between species. Previous studies on seedling growth (Rodríguez-Calcerrada et al., 2008) found that when there was no water shortage, *Q. petraea* seedlings grow more than those of *Q. pyrenaica* in greater light conditions, being those differences minimal when both species were in shade. In contrast, our study showed that *Q. pyrenaica* growth was greater than *Q. petraea* in all conditions (i.e. open areas and under shrubs), pointing out the higher stress undergone by *Q. petraea* compared with *Q. pyrenaica* in this reclaimed site. The limiting conditions of the summer drought probably affected more *Q. petraea*, with more drought-sensitive features, than *Q. pyrenaica*, better adapted to water stress (Rodríguez-Calcerrada et al., 2008), reducing its growth and the effectiveness of its reintroduction in these mined sites compared with *Q. pyrenaica*.

4.3. Acorn emergence and survival

Mesh protection prevents acorn predation by small mammals, a common problem for *Quercus* regeneration even if acorns are buried (Gómez et al., 2003; Smit et al., 2008). Mesh protection was a very important factor for acorn emergence in our experiment, evidenced by the higher emergence rates in meshed plots compared with no-mesh plots in the mine. In the same way, shrubs influenced positively acorn emergence of both species. It is well known that acorn germination in *Quercus* species is conditioned by soil moisture (Pérez-Ramos et al., 2013), and shrubs may prevent acorn desiccation improving acorn germination and emergence rates (Smit et al., 2008). Moreover, acorns of *Quercus* species may present delayed emergence, which happens when germination occurs late and the summer drought inhibit their growth until moisture conditions become optimal again (González-Rodríguez et al., 2012). Then, it is possible that at the end of spring soil moisture in mined areas decreased faster than in the forest, mainly by its lower water holding capacity, delaying the emergence of acorns in time until the next autumn rainfall, when emergence in mined areas experienced a visible increase. According to the observed emergency pattern, it seems that acorns are better seeded as soon as possible in autumn, if the risk of waterlogging is low; thus, acorns have more possibilities to use water resources for their development (Urbieta et al., 2008).

Mesh protection in the mine was also important for seedling survival after emergence because rodents can predate acorns even when they are attached to the seedling root, killing the seedling (Gómez et al., 2003). In addition, emerged seedlings did not seem to depend on shrub influence, probably because during the first year seedlings have a strong dependence on cotyledon reserves, being less influenced by environmental conditions (Pérez-Ramos et al., 2010). Both facts can explain the trend found in acorn survival for the mesh treatment. As the number of emerged acorns increased with time, being greater for *Q. petraea*, those individuals

were included in the survival values. Acorn survival values did not experience a noticeable decrease because once acorns were protected from predators the environmental conditions did not influence acorn survival negatively. Overall, only seedling survival in mine plots with shrubs and mesh was similar to forest plots, pointing out the importance of shrubs in restoration for achieving the same dynamics of emergence and seedling survival than in natural forest systems.

5. Conclusions

The positive effects of shrubs upon seedling survival and growth and acorn emergence found in this study showed that the use of natural colonizer shrubs (*C. scoparius* and *G. florida*) as nurse plants is promising for the effective reintroduction of late successional tree species, such as *Q. petraea* and *Q. pyrenaica*, in reclaimed coal mines. However, two key parameters should be taken in consideration; (i) the ontogenetic stage of the target species introduced (i.e. acorn or seedling) in order to avoid problems during establishment, and (ii) the stress tolerance strategy of the selected species; i.e. the success being greater if the species is less sensitive to the main constraints in the area.

Here, the best method for tree species reintroduction was obtained by using *Q. pyrenaica* seedlings under shrubs. This method avoids problems such as acorn predation and germination failure, and *Q. pyrenaica* has been shown to be better adapted to water stress than *Q. petraea* in the study area. In contrast, if we are interested in seeding, acorns of *Q. petraea* beneath shrub canopies and protected from rodents would be the best method. Nevertheless, other alternative seeding techniques should be tested before ruling out seeding as option to *Quercus* species reintroduction in Mediterranean mined areas. Finally, further investigations are needed to assess the effectiveness of this technique in the long-term. It would be necessary to monitor the interactions between shrubs and target species to detect life-stage conflicts mediated by competition for resources or environmental conditions that could arise when seedlings grow taller, as well as the possible effect of wild ungulates and/or livestock browsing on survival and growth of taller seedlings, despite not being a major constraint at early stage.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at <http://dx.doi.org/10.1016/j.ecoleng.2015.01.024>.

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