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Reintroduction of *Quercus petraea* and *Quercus pyrenaica* mediated by nurse shrubs on reclaimed coal wastes in Northern Spain

Alumna: Paloma Torroba Balmori
Tutora: Carolina Martínez Ruiz

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Índice

0. ABSTRACT

Facilitative interactions among plants enable a species persist in severe environments, but its use as a restoration technique of plant cover in degraded man-made ecosystems requires more accuracy. Thus, a 2-year field experiment was conducted to determine if nurse shrubs (*Genista florida* and *Cytisus scoparius*) would benefit *Quercus petraea* and *Q. pyrenaica* reintroduction in a reclaimed coalmine in Northern Spain. 400 seedlings and 940 acorns of each species were planted under different treatments, combining presence and absence of both shrubs and herbivore pressure, and emergence, survival and growth were monitored. The results showed that shrubs enhanced seedling survival (more than 4-higher) and growth, as well as acorn emergence, reducing the effect of the summer drought, the main constrain in the area (survival <20% in open areas, >90% under shrubs after the first summer). Acorn predation was also a problem, but not seedling herbivory. As the life stage and the stress tolerance of the species also influenced the results, being better in case of *Q. pyrenaica* seedlings, reintroduction of forest species in mined sites should consider the influence of nurse shrubs, the life stage and the stress tolerance strategy of the target species in order to optimize the restoration.

KEY WORDS: *Facilitation, restoration, abiotic stress, herbivory, Mediterranean environment, shrub cover.*

0. RESUMEN

Las relaciones de facilitación entre plantas permiten a una especie persistir en ambientes difíciles, pero su uso como técnica de restauración de la cubierta vegetal en ecosistemas antrópicos degradados necesita más conocimiento. En este sentido, se realizó un experimento de campo durante dos años para determinar si los arbustos nodriza (*Genista florida* y *Cytisus scoparius*) beneficiarían la reintroducción de *Quercus petraea* y *Q. pyrenaica* en una mina de carbón restaurada en el norte de España. Se plantaron 400 plántulas y 940 bellotas de cada especie bajo diferentes tratamientos que combinaban la presencia/ausencia de arbustos y la presión de herbivoría, y se realizó un seguimiento de la emergencia, supervivencia y crecimiento. Los resultados mostraron que los arbustos mejoraban la supervivencia de las plántulas (más de 4 veces) y el crecimiento, así como la emergencia de las bellotas, reduciendo el efecto de la sequía estival, el principal problema en la zona (supervivencia <20% en áreas abiertas, >90% bajo arbustos después del primer verano). También hubo problemas de depredación sobre bellotas pero no sobre plántulas. Como la edad y la tolerancia al estrés de la especie también afectaron a los resultados, siendo mejores para plántulas de *Q. pyrenaica*, la reintroducción de las especies forestales en áreas mineras debería considerar la influencia de los arbustos nodriza, la fase de vida y la tolerancia al estrés de la especie a introducir para optimizar la restauración.

PALABRAS CLAVE: *Facilitación, restauración, estrés abiótico, herbivoría, ambiente mediterráneo, cobertura arbustiva.*

1. INTRODUCTION

Facilitation in plant communities encompasses non-trophic, beneficial interactions between organisms (Brooker *et al.*, 2008). Facilitation is achieving its own field research due to its importance in the understanding of ecological dynamics, ecosystem restoration, and its potential evolutionary impact, among other topics (Brooker and Callaway, 2009). However, there is still much to understand about how facilitative interactions work (Brooker *et al.*, 2008), especially in novel ecosystems created as a consequence of ecological restoration of man-made impacts (Hobbs *et al.*, 2009).

The plant-plant interaction outcome is a complex process which depends on many factors, among others species identity and the severity of the environment (Gómez-aparicio *et al.*, 2004), the life stage of the interacting organisms and plant traits of neighbours (Padilla and Pugnaire, 2006; Butterfield, 2009). Conceptual models explaining facilitation (see Bertness and Callaway, 1994, for the Stress-Gradient Hypothesis; and Butterfield, 2009, for the hump-shaped pattern) do not still represent the reality of the processes properly (Brooker *et al.*, 2008). Despite the fact that 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 (Gómez-Aparicio, 2009), like in degraded ecosystems (e.g. mined sites). In fact, facilitation processes enable a species to enlarge its niche space, persisting in severe environments where it is usually absent when isolated (Bruno *et al.*, 2003). Recent studies have suggested to apply facilitation processes in restoration ecology, as a tool to enhance plant performance in disturbed environments, where harsh environmental conditions or herbivory are major constraints (Padilla and Pugnaire, 2006; Brooker *et al.*, 2008). However, an efficient use of this ecological mechanism in restoration requires more accuracy about the results of particular target and nurse species interactions under specific problematic conditions, to ensure the viability of this technique. In order to achieve so, widening the range of species and environments considered in the experiments seems necessary.

In the Iberian Peninsula, most of the research on this topic has been carried out in natural semi-arid ecosystems of Southwest Spain (Pugnaire *et al.*, 2011). However, few field experiments are carried out testing facilitation processes and its usefulness as a restoration technique in man-made ecosystems, such as open-cast coal mines. Coal mining is an extended activity in Northwest of Spain (Alday *et al.*, 2011), where *Quercus petraea* (at its southernmost distribution limit; Blanco Castro *et al.*, 2005) and *Quercus pyrenaica* (almost endemic of Iberian Peninsula; do Amaral Franco, 1990) are major forest species. The restoration of these native forest species is very important for the maintenance of ecological functions, biodiversity and landscape values (Blanco Castro *et al.*, 2005).

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 of broadleaf species cover present before the mining operations (tree species from target community), such as *Q. petraea* and *Q. pyrenaica,* in these mine sites is a hard task. It is well known that in Mediterranean ecosystems, forest species regeneration suffers from two major constrains: (i) summer drought (Castro *et al.*, 2004, Gómez-Aparicio *et al.*, 2008), which effect in reclaimed mined sites is increased by a lack of developed soil such as in forest systems (Martínez-Ruiz and Marrs, 2007; 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, Zamora *et al.,* 2001). In order to overcome these two constraints, facilitation mediated by shrubs has been proposed for the reforestation of Mediterranean systems (Gómez-Aparicio *et al.*, 2004; Castro *et al.*, 2006) which could be also implemented for mining sites. 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 (García and Obeso, 2003). Thus, in the present study we assessed the influence of nurse shrubs through different seasons upon reforested seedlings and acorns of two *Quercus* species (*Q. petraea*, *Q. pyrenaica*) in an open-cast coal mine, in order to reintroduce the tree species present before the mining operations.

2. OBJECTIVES

The main objective was to assess the influence of nurse shrubs in the reintroduction of *Q. petraea* and *Q. pyrenaica* in an open-cast coal mine, testing their effect against the main problems suffered by forest species regeneration in Mediterranean ecosystems: summer drought and herbivory.

The hypotheses to test were:

1) The effect of shrubs and enclosures would be positive for survival of seedlings of the two *Quercus* species.
2) Shrubs and enclosures would be also beneficial for seedling growth.
3) Emergence and survival of acorns of the two *Quercus* species would be positively affected by shrubs and mesh protection.

3. MATERIAL AND METHODS

3.1. Site description and mine restoration treatment

The experiment was located in a 7 ha reclaimed open-cast coal mine near Guardo, Palencia, Northern Spain (lat 42° 48′ N; long 4° 52′ W, ca. 1200 m a.s.l.). The climate is sub-humid Mediterranean (Alday *et al.*, 2011), with an annual mean temperature of 9.3 ºC 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; most rain falls during spring and autumn and there is a 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), affecting the performance of our species throughout the time (data from Cervera meteorological station, 30 km away from Guardo, due to the closure of Guardo meteorological station). The vegetation surrounding the mine consisted of broad-leaved woodlands dominated by *Quercus pyrenaica* and *Quercus petraea*, and some shrubs such as *Cytisus scoparius* and *Genista florida*.

After coal mining stopped, the open pit was filled with coal wastes from nearby mines and the surface was covered with ca. 30 cm of fine-textured sediments and a layer of cattle manure (10 t ha^{-1}). Thereafter, in October 2000 the entire site was hydroseeded with grasses and herbaceous legumes by the mining company (Unión Minera del Norte S.A.; for further reclamation details see: Alday *et al.*, 2011). Nowadays, over the reclaimed area there are herbaceous plants and patchy natural colonization spots of shrubs, mainly *Cytisus scoparius* and *Genista florida*, being grazed freely by wild animals from the surrounding landscape (deer, roe deer, board), 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).

3.2. Experimental design and data collection

3.2.1 Seedlings experiment

The experimental set-up consisted of four main-plots, (30×30 m, flat area), which combined the influence of nurse shrubs and grazing upon *Quercus* seedlings (2 fenced main-plots and 2 no-fenced main-plots, all of them including shrub cover and open areas). 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 cover (no-fenced open areas, ON); (c) grazing and shrubs cover (non-fenced areas under shrubs, SN). The species used as nurse shrubs were *Cytisus scoparius* and *Genista* *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 fix the atmospheric nitrogen (N_2) (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 same areas (Ruiz de la Torre, 2006), as it happens in the study area, where they regenerate very mixed. As a consequence and based in 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 plots.

Within each main-plot, ten sub-plots were allocated randomly ca. 4 m apart from each other (each sub-plot ca. 2 × 2 m; when sub-plots were under shrubs they gathered 2-3 shrubs), where the optimal conditions for each treatment were found. In each sub-plot 10 tagged seedlings (one-year-old) of each species (400 seedlings of *Q. petraea* and *Q. pyrenaica*) were planted in March 2011. Seedlings (Cordillera Cantábrica Provenance), grown in cylindrical pots (type: S.L 35, 235 cm^3, 16 cm deep × 5 cm wide) filled with peat, were provided by the central nursery of Junta de Castilla y León, in Valladolid**.** They were placed systematically 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. When the lowest branches of the shrub prevented the seedlings 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 × 6 cm) to minimize the damage to the shrub roots and soil structure disturbance. In all 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 than in 2012 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 the time for those seedlings apparently dead which resprouted in the next surveys.

3.2.2. Acorn experiment

A sowing experiment was performed to study the influence of shrubs and herbivore protection upon emergence of acorns and survival of those seedlings. In November 2011 we collected *Q. petraea* and *Q. pyrenaica* acorns from nearby forests and seeded them in two treatments in the mine: under shrubs (n=25 plots) and in open areas (n=25 plots). In each plot (1 x 0.25 m), 20 acorns of each species were buried horizontally into the soil at 2-3 cm (40 acorns per 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). Non-viable acorns (empty or preyed upon by insects) were excluded by floating in water (Gómez *et al.*, 2003). In order to control the acorn performance in the forest, we also set up plots in the forest (n=10 plots; 1 × 0.25 m).

In March 2012, some plots were altered by animals and humans. Some wire meshes disappeared and in a few plots the soil surface was dug by wild boards. Therefore, we excluded destroyed plots changing the experimental design so that we could analyse the effect of environmental conditions and protection against rodents (final number of plots and acorns: 47 plots, 940 acorns per species). Thus, the treatments considered were: in the mine (1) under shrubs with mesh (SM; n=17 plots), (2) open areas with mesh (OM, n=7), (3) under shrubs without mesh (SN, n=5), (4) open areas without mesh (ON, n=13); (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.

Previously to this experiment, another seeding experiment was carried out in March 2011, with the same experimental set-up that for seedling experiment, where acorns were seeded into wire mesh cages. Rodent predation was very high and no acorn survived after 5 months, thus results are not showed here.

3.3. Data analysis

All statistical analyses were implemented in the R software environment (2.15.2; R Development Core Team, 2012). Seedlings and acorns survival were analyzed using lme4 package for Generalized 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 analyzed, 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 analyze the effect of shrub, mesh, site (mine or forest) 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/plot).

The survival 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 only considered the seedlings which reached alive the end of the second growing season. The effect of shrub, fence 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.

4. RESULTS

4.1. Seedling survival

The GLMM showed that there was a significant shrub×time×species interaction (Table 1a). However, fenced areas (SF, OF) had no significant effect on seedlings survival. Seedling survival descended through the time for both species, but *Q. pyrenaica* survival was always greater than *Q. petraea* survival (Fig. 1). At the same time, the survival for both species was greater under shrubs than in open areas. In open areas seedling mortality 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 6th and 16th month after plantation: *Q. petraea*=39.7%, *Q. pyrenaica*=30.6%, Fig. 1). Thus, in the first autumn, 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 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%).

Table 1. Parameters estimated by the GLMM model for seedling survival (Table 1a, *Z* values) and for seedling growth (Table 1b, *t* values). O-Pt: *Q. petraea* in open areas.

Fixed effects	Estimate±S.E.	value	*p*-value
a) seedling survival			
Intercept_O-Pt	5.39±0.60	9.03	<0.001
shrub	0.12±0.80	0.15	0.880
time	-1.40±0.26	-5.40	<0.001
Q. pyrenaica	0.24±0.24	0.97	0.330
shrub × time	1.33±0.27	4.87	<0.001
shrub × *Q. pyrenaica*	0.45±0.50	0.91	0.364
time × *Q. pyrenaica*	0.16±0.04	4.45	<0.001
shrub × time × *Q. pyrenaica*	-0.17±0.04	-3.72	<0.001
b) seedling growth			
Intercept_O-Pt	2.14±1.01	2.11	0.036
shrub	5.69±1.03	5.52	<0.001
time	-0.65±0.10	-0.65	0.519
Q. pyrenaica	1.30±0.35	3.72	<0.001
shrub × time	-4.06±1.06	-3.82	<0.001

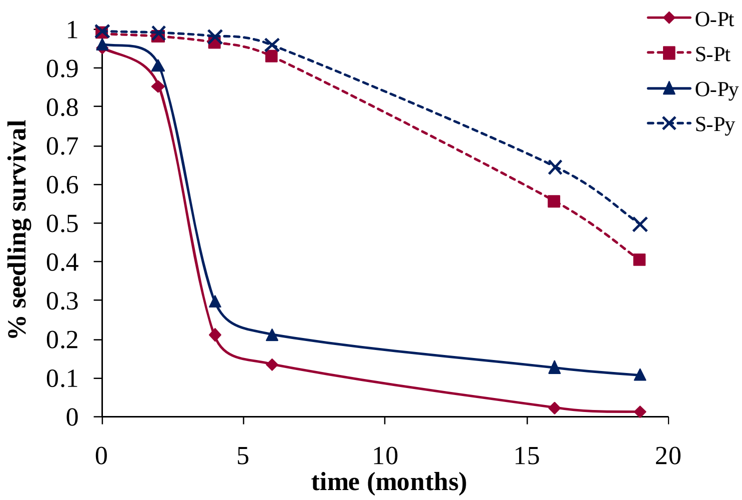

Fig. 1. Seedling survival of *Q. petraea* and *Q. pyrenaica* species in open areas and under shrubs through the two growing season. The fitted line represents the minimal adequate GLMM. O-Pt: *Q. petraea* in open areas (n=20 plots, 200 individuals in total), S-Pt: *Q. petraea* under shrubs (n=20 plots, 200 ind. in total), O-Py: *Q. pyrenaica* in open areas (n=20 plots, 200 ind. in total), S-Py: *Q. pyrenaica* under shrubs (n=20 plots, 200 ind. in total).

4.2. Seedling growth

Growth performance was analyzed only in 27.8% of the seedlings, 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 species under shrubs grow 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 the second year regarding the previous year (first year: 8.3 ±0.5cm *vs*. second year: 2.9±0.3cm for *Q. petraea*; first year: 8.9±0.5cm *vs*. second year: 4.7±0.3cm for *Q. pyrenaica*; Fig. 2). It is noteworthy to mention that the species had a significant effect on growth, since *Q. pyrenaica* seedlings grew more than those of *Q. petraea* ($F_{[1,\ 196]}$=13.87, p<0.001; Table 1 and Fig. 2).

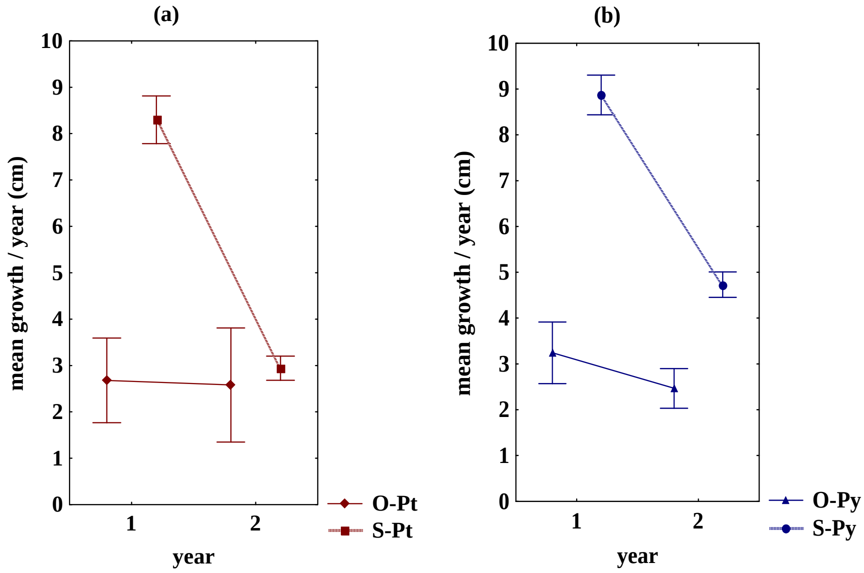

Fig. 2. Seedling mean growth of a) *Q. petraea* and b) *Q. pyrenaica* species in open areas and under shrubs at the end of each growing season. Bars represent the standard error. O-Pt: *Q. petraea* in open areas (n=2 plots, 5 individuals in total), S-Pt: *Q. petraea* under shrubs (n=18 plots, 91 ind. in total). O-Py: *Q. pyrenaica* in open areas (n=6 plots, 21 ind. in total). S-Py: *Q. pyrenaica* under shrubs (n=18 plots, 105 ind. in total).

4.3. 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±5.7%, OM=21.4±6.6%, ON=4.6±2.4%, SN=0%; *Q. pyrenaica*: SM=21.2±4.0%, OM=12.9±4.2%, ON=3.5±1.6%, SN=1.0±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). Also, *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, GLMM results showed that although the emergence values were stable in forest area for both species in time (*Q. petraea*: 30-38%, *Q. pyrenaica*:

≈10%, Table 2b, Fig. 3a), showing *Q. petraea* significantly greater values than *Q. pyrenaica*, in the mined areas the emergence probability increased with time for both species and the difference in emergence among them was slightly lower (Fig. 3a).

Table 2. Parameters estimated by the GLMM model (*Z* values) for acorn emergence in mine areas (Table 2a, ON-Pt: *Q. petraea* in open areas without mesh) and for acorn emergence in mine areas with mesh and the forest (Table 2b, FM-Pt: *Q. petraea* in forest areas with mesh).

Fixed effects	Estimate±S.E.	*Z* value	*p*-value
a) acorn emergence in mine areas			
Intercept_O-Pt	-3.90±0.51	-7.69	<0.001
mesh	0.86±0.16	5.40	<0.001
shrub	0.06±0.61	0.10	0.924
Q. pyrenaica	-0.64±0.15	-4.35	<0.001
shrub × *Q. pyrenaica*	-0.47±0.18	-2.66	0.008
b) acorn emergence in mine and forest			
Intercept_FM-Pt	-1.89±0.71	-2.67	0.008
mine _mesh	-1.37±0.57	-2.38	0.017
Q. pyrenaica	-1.83±0.22	-8.47	<0.001
time	0.02±0.02	1.18	0.239
mine _mesh × *Q. pyrenaica*	0.85±0.23	3.63	<0.001
mine _mesh × time	0.03±0.02	2.05	0.041

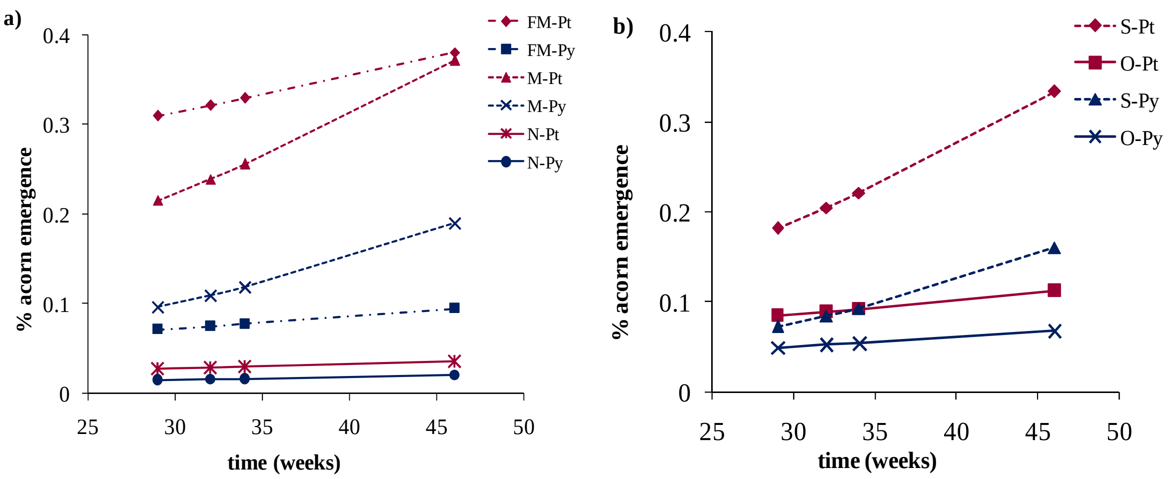

Fig. 3. a) Probability of acorn emergence of *Q. petraea* and *Q. pyrenaica* species in the forest with mesh and in the mine areas with mesh and without mesh through the growing season. FM-Pt: *Q. petraea* in the forest with mesh (n=5 plots, 100 acorns in total), FM-Py: *Q. pyrenaica* in the forest with mesh (n=5 plots, 100 acorns in total), M-Pt: *Q. petraea* in areas with mesh (n=24 plots, 480 acorns in total). N-Pt: *Q. petraea* in areas without mesh (n=18 plots, 360 acorns in total), M-Py: *Q. pyrenaica* in areas with mesh (n=24 plots, 480 acorns in total), N-Py: *Q. pyrenaica* in areas without mesh (n=18 plots, 360 acorns in total). 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 (n=22 plots, 440 acorns in total). O-Pt: *Q. petraea* in open areas (n=20 plots, 400 acorns in total), S-Py: *Q. pyrenaica* under shrub (n=22 plots, 440 acorns in total), O-Py: *Q. pyrenaica* in open areas (n=20 plots, 400 acorns in total). For both figures a) and b), the fitted line represents the minimal adequate GLMM.

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±4.9%, OM=2.1±1.0 %, SN=0%, ON=0%, and for *Q. pyrenaica*: SM=15.6±3.7%, OM=0.7±0.7%, SN=0%, ON=1.9±1.6%. In the mine, the survival of acorns seedlings was influenced by mesh×time×species interaction (Table 3a, Fig. 4). The survival was greater in areas covered with mesh (plots with mesh: 14.5±2.7%, plots without mesh: 0.7±0.6%; mean±S.E.), increasing significantly with time in *Q. pyrenaica* and decreasing in *Q. petraea*, but always being greater in *Q. petraea* than in *Q. pyrenaica* (*Q. petraea* with mesh: 17.7±4.0%, *Q. pyrenaica* with mesh: 11.3±2.9%). Moreover, there was no difference in species survival between forest microhabitat and the treatment SM and SN (Table 3b), but survival in the other treatments in the mine was lower compared with the forest (Table 3b). Nevertheless, the results of SN should be considered with precaution, because this treatment also had a much lower value of survival than the forest microhabitat, but differences were not found because of its large standard error (Table 3b).

Table 3. Parameters estimated by the GLMM model (*Z* values) for acorn survival in mine areas through the time (Table 3a, ON-Pt: *Q. petraea* in open areas without mesh) and for acorn survival among mine areas and forest at the end of the growing season (Table 3b). FM-forest sites with mesh (n=5 plots, 200 acorns in total), ON-mine open areas without mesh (n=13 plots, 520 acorns in total) OM-mine open areas with mesh (n=7 plots, 280 acorns), SN-mine areas under shrubs without mesh (n=5 plots, 200 acorns in total), SM-mine areas under shrubs with mesh (17 plots, 680 acorns).

Fixed effects	Estimate± S.E.	*Z* value	*p*-value
a) through the time			
Intercept_O-Pt	4.85±3.37	1.44	0.149
mesh	-4.53±3.33	-1.36	0.174
time	-0.28±0.11	-2.57	0.010
Q. pyrenaica	-10.48±3.54	-2.96	0.003
shrub	0.23±0.68	0.34	0.733
mesh × time	0.18±0.11	1.68	0.093
mesh × *Q. pyrenaica*	8.37±3.58	2.34	0.019
time × *Q. pyrenaica*	0.31±0.11	2.77	0.006
mesh × time × *Q. pyrenaica*	-0.28±0.11	-2.46	0.014
b) the end of the growing season			
Intercept_FM	-1.65±0.23	-7.15	<0.001
ON	-3.04±0.52	-5.84	<0.001
OM	-2.63±0.58	-4.57	<0.001
SN	-16.94±782.39	-0.02	0.983
SM	0.24±0.21	1.12	0.262

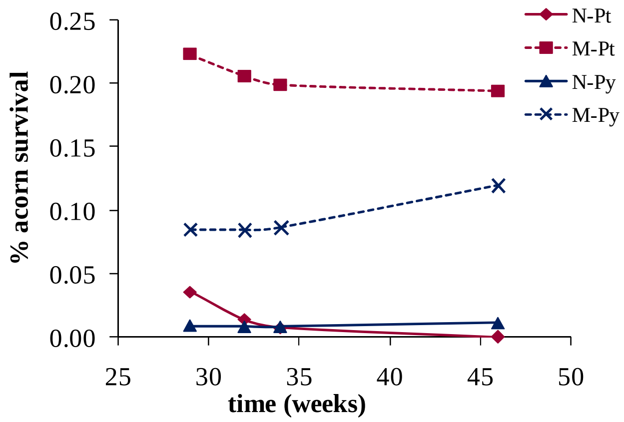

Fig. 4. Probability of acorn survival of *Q. petraea* and *Q. pyrenaica* species in mine areas with mesh and without mesh through the growing season. The fitted line represents the minimal adequate GLMM. M-Pt: *Q. petraea* in areas with mesh (n=24 plots, 480 acorns in total). N-Pt: *Q. petraea* in areas without mesh (n=18 plots, 360 acorns in total), M-Py: *Q. pyrenaica* in areas with mesh (n=24 plots, 480 acorns in total), N-Py: *Q. pyrenaica* in areas without mesh (n=18 plots, 360 acorns in total).

5. DISCUSSION

Reintroduction of *Quercus* seedlings and acorns was influenced by microsite characteristics, achieving better results under shrubs than in open areas, whereas protection against herbivory only resulted important for acorns. The life stage and the stress tolerance of the species also determined the results of *Quercus* reintroduction.

5.1. Seedling survival

Our data show that the shrubs enhance seedling survival, as expected, compared with seedlings planted in open areas. After two years, the survival under shrubs for *Q. petraea* and *Q. pyrenaica* was 17.8- and 4.7- higher respectively. However, fenced areas did not enhance seedling survival as expected, which suggest mammal herbivory is not a major problem for *Quercus* regeneration in the area, contrary to other results found in different regions of Spain (Zamora *et al.*, 2001; García and Obeso, 2003; Gómez *et al.*, 2003). Although, *Quercus* regeneration problems caused by mammal herbivores have been reported in other studies (Kuiters and Slim, 2002; Bakker *et al.*, 2004), *Quercus* species are not very attractive for some herbivores, like cattle, due to their high tannin concentrations (Rodríguez-Doce, 2010). Here our results are in agreement with this, since the ungulates impact to oak regeneration has been residual; probably thanks to the presence of available forage of higher quality in the mine site composed mainly by herbaceous legumes and grasses (López Marcos, 2012).

As expected on Mediterranean systems, the first summer after plantation was a critical period for seedling survival (Castro *et al.*, 2004; 2006; Gomez-Aparicio *et al.*, 2005a,b), since 48.6% of seedlings died between May and September 2011. However, during the first dry season the most affected individuals were those mainly located in open areas, in contrast most of seedlings under shrubs survived after that period, pointing out the positive effect of shrubs on seedling survival when conditions became worse. On the other hand, mortality of seedlings under shrubs increased during the second year after plantation. In this experiment, the lower precipitations in the 2011-2012 period than in the 2010-2011 one probably reduced the supplies of soil water in the mine soil, producing very hard conditions for vegetation survival in the mine at the latter period. Especially if we consider that the water holding capacity of mine soil is clearly lower than in the nearby natural forest soil (1-3.5 g/cm^2 *vs*. 19.8 g/cm^2; López Marcos, 2012). Then, it looks like that amelioration of environmental conditions by shrubs was not enough to satisfy the requirements of our seedlings during the more severe period (2011-2012). Thus, although seedlings can survive better under shrubs, under extreme dry seasons canopy cover is not enough to prevent the death effect on seedlings. Similar studies have documented that extremely high 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 outcome among plants to negative when water resources decrease; possibly because the facilitator cannot compensate for its own resource competition or rain interception (Tielbörger and Kadmon, 2000; Maestre and Cortina, 2004).

In general, it seems that a positive effect on seedling survival exists when seedlings are set under shrubs, possibly due to the result of the interaction among shrubs and seedlings. The positive interactions between shrubs and 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), and support the idea of using natural processes as restoration treatments, which allow to obtain a self-sustaining system in the long term at a low cost (Bradshaw, 1997). Here, the effect of shrubs in the reduction of light levels (Rey-Benayas *et al.*, 2002, Smit *et al.*, 2008) and the improvement of soil properties (e.g. fertilizing, water holding capacity or nitrogen fixing; Pugnaire *et al.*, 2004; Gomez-Aparicio *et al.*, 2005a,b), 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. At the same time, without manipulation of shrub cover (see Gómez-Aparicio *et al.*, 2005b), we cannot rule out that the observed effect is also produced by other variables (Tielborger and Kadmon, 2000, Frouz *et al*., 2011), e.g. starting soil quality that could have been better in some places of the mine, resulting in the establishment and development of shrub vegetation.

The different patterns of species survival 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 badly 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.*, 2008a). Those differences may explain why *Q. pyrenaica* has shown higher survival throughout the time compared with *Q. petraea* in our experiment, such as it happens in other studies in Mediterranean habitats (Rodríguez-Calcerrada *et al.*, 2008b, 2010). Therefore, it seems necessary to consider each species strategy and characteristics in order to determine the most adapted to the main mine-site constrains. This species selection will determine the success of trees 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).

5.2. Seedling growth

It was expected that shrubs and fenced areas improved seedling growth but, just like it happened with seedling survival, shrubs had a positive effect on growth whereas enclosures did not affect it.

The higher growth found in seedlings under shrubs is probably caused by less stressful conditions in light and soil properties and better water status than seedlings in open areas, which also resulted in higher growth. These results contrast with other studies (Marañon *et al.*, 2004; Gómez-Aparicio *et al.*, 2005a), where despite finding higher seedling survival in shady habitats, under neighbour plants the growth decreased compared with seedling in 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 on occasion, 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; Marañon *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, similarly to seedling survival, seedling growth under shrubs decreased regarding the first year. In addition, we found differences in growth between species, as it also happened in seedling survival. Previous studies (Rodríguez-Calcerrada et al., 2008a) found that when there was no water shortage, *Q. petraea* seedlings tended to grow more than those of *Q. pyrenaica* in high light conditions, being those differences minimal when both species were in shade. However, in our study, *Q. pyrenaica* showed higher growth in all conditions, open areas and under shrubs, pointing out the higher stress undergone by *Q. petraea* compared with *Q. pyrenaica*. When temperate tree species like *Q. petraea* grow in Mediterranean habitats, drought usually arises as the principal limiting factor (Rodríguez-Calcerrada et al., 2008a). The limiting conditions of the summer drought probably affected more to *Q. petraea*, with more drought-sensitive features, than to *Q. pyrenaica*, better adapted to water stress (Aranda et al., 2004; Rodríguez-Calcerrada et al., 2008a,b), reducing its growth and the effectiveness of the reintroduction compared with *Q. pyrenaica* despite the benefits of shrubs microsites.

5.3. Acorn emergence and survival

Our data show that mesh protection was important to prevent acorn predation by small mammals, a common problem for *Quercus* regeneration even if acorns are buried (Gómez *et al.*, 2003), evidenced by the higher emergence rates in meshed microsites compared with non-mesh microsites in the mine. Similarly, shrubs influenced positively acorn emergence of both species in the mine. Soil moisture is a very important factor for acorn germination in *Quercus* species (Pérez-Ramos *et al.,* 2013), and shrubs can prevent their desiccation improving acorn germination and emergence rates (Smit *et al.*, 2008). On the other hand, 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 (Gómez, 2004; 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, because of its soil characteristics and less vegetation cover, delaying the emergence of acorns in time until autumn rainfall, when emergence in mined areas experienced a visible increase. Time and rates of emergence also depend on levels of light, seed mass and maternal plant, and their influence vary among species (Marañon *et al.*, 2004; Urbieta *et al.*, 2008; González-Rodríguez *et al.*, 2012), which may explains the different rates of emergence achieve by our *Quercus* species. For example, *Q. pyrenaica* may present higher germination and emergence in full light and medium-shade habitats (under shrubs or isolated trees) than in the forest (Marañon *et al.*, 2004), whereas soil moisture is a major factor influencing *Q. petraea* germination (Kollmann and Schill, 1996), which could explain why the emergence rates of *Q. pyrenaica* were slightly higher in the mine than in the forest, contrary to *Q. petraea*.

Mesh protection in the mine was also important for seedling survival after germination, because rodents can predate acorns even when they are attached to the seedling root, killing the seedling (Gómez *et al.*, 2003). On the other hand, emerged seedlings did not seem to depend on shrub influence, probably because during the first year seedlings have a strong dependence on cotyledon reserves and are less influenced by environmental conditions (Pérez-Ramos *et al.*, 2010). Despite this relative independence of environment, *Q. petraea* survival decreased through the time whereas *Q. pyrenaica* trend was to survive after emergence. This behaviour could be related to their different life strategies, since *Q. pyrenaica* is more stress tolerant than *Q. petraea* (Rodríguez-Calcerrada *et al.*, 2008a). Overall, only seedling survival in mine microsites with shrubs and mesh was similar to forest microsites, pointing out the importance of shrubs in restoration for achieving the same dynamics of germination and seedling survival than in natural systems and highlighting the problem of acorn predation.

6. CONCLUSIONS

Our results show that the use of shrubs as nurse plants is beneficial for reintroduction of *Q. petraea* and *Q. pyrenaica* in mined sites, ameliorating the hard conditions of summer drought, the main constrain. This positive interaction is recognised as a facilitation process, ecologically important in degraded systems (Gómez-Aparicio, 2009), and considered as a restoration treatment in ecological engineering (Bradshaw 1997). The positive effects of shrubs upon germination, survival and growth contrast with other studies, which found opposite effects on survival and growth (Gómez-Aparicio *et al*., 2005a), and on seed and seedling stages (Smit *et al*., 2008). Overall, the present work shows that reintroduction of forest species in mined sites should consider the positive influence of nurse shrubs, the life stage of the target species in order to avoid problems during establishment, and the stress tolerance strategy of the target species, the success being higher if the species is less sensitive to the main constrains in the area. In our case, the best results in reintroduction would be obtained through *Q. pyrenaica* seedlings under shrubs, which would avoid problems such as acorn predation and germination failure and would be better adapted to water stress than *Q. petraea*. To determine the final viability of this technique would be necessary monitoring the interactions among shrubs and target species in the long term, in case life-stage conflicts (sensu Schupp, 2007) mediated by competition for resources or environmental conditions that could arise when seedlings grew larger. In addition, other alternative seeding techniques should be tested before rule out seeding as option to *Quercus* spp. reintroduction.

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