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Can native shrubs facilitate the early establishment of contrasted co-occurring oaks in Mediterranean grazed areas?

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Nomenclature
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Abstract

Questions: Can shrubs (*Cytisus multiflorus*) and large herbivore exclusion (fence) facilitate seedling survival and growth of marcescent and sclerophyllous oaks (*Quercus pyrenaica* vs *Q. ilex* subsp. *ballota*) under a bioclimatic limit in Mediterranean grazed areas?

Location: Open oak woodlands, central-western Spain.

Methods: A 2-yr field experiment was conducted by planting 200 seedlings of each *Quercus* species under four different treatments combining the influence of nurse shrubs and fencing on *Quercus* seedling survival and growth.

Results: *Cytisus multiflorus* enhanced poor *Quercus* seedling survival found in the study area, at least during the first 2 yr after planting and particularly during the first dry season. The improvement in soil organic matter under the shrub canopy may have contributed to this positive effect, which was more pronounced on *Q. pyrenaica* seedlings. Seedling herbivory did not seem to be a limitation to survival. Increased seedling growth in both species was also very low, and no growth was recorded 2 yr after planting without shrubs. The positive shrub effect on seedling growth, especially marked in fenced areas, was more important in *Q. pyrenaica* in the first growing period and in *Q. ilex* in the second; 2 yr after planting no difference in shrub effect on growth was found in either *Quercus* species. Seedling herbivory was a limitation to seedling growth in areas without shrubs, mainly in the case of *Q. pyrenaica*.

Conclusions: In Mediterranean grazed areas with important summer drought and very sandy soil, shrubby *C. multiflorus* plants have a clear facilitative effect on seedlings of ecologically contrasted *Quercus* species. The facilitative effect was found in both marcescent and sclerophyllous oak seedlings, but to a different degree depending on the species considered and the variable measured (survival or growth). In terms of survival, the marcescent species was more favoured by shrub cover than the sclerophyllous one, and this effect was accentuated through time. However, in terms of growth, although *Q. pyrenaica* was initially more favoured by shrubs, differences between the two species were attenuated after 2 yr. Therefore, *C. multiflorus* can have a key role in restoration of these oak degraded environments.

Introduction

Many oak woodlands worldwide have been transformed by livestock farming, giving rise to savanna-like landscapes (McCreary 2004; Bergmeier et al. 2010) with a predominant pasture matrix of isolated oak trees and shrubs, with more or less cover often depending on the stocking rate. These landscapes present high biological diversity associated with the high structural diversity generated (Díaz et al. 1997; Rolo et al. 2013), and oak trees are essential for the maintenance of ecosystem services (Marañón et al. 2009). However, these woodlands are suffering dieback of

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adult trees (Pulido et al. 2001) and low regeneration rates mainly caused by deficient seed dispersal to suitable sites for seedling establishment and survival (Pulido & Díaz 2005). Moreover, reforestation with *Quercus* species tends to be difficult due to their low rates of growth and survival, particularly when conditions of the first dry season are very stressful (Navarro-Cerrillo et al. 2005). It is therefore of particular interest to identify effective 'safe sites' for tree regeneration in these systems.

Several studies have shown that shrubs may play a major role in oak regeneration (Gómez et al. 2008; Pulido et al. 2010; Rolo et al. 2013). Two decades ago, Bertness & Callaway (1994) proposed that strong positive interactions should be dominant over competition, as the net outcome of simultaneous interactions, in communities developing under high abiotic stress or high consumer pressure. Since then, numerous empirical studies have reported that positive interactions are the rule under severe physical conditions (Callaway 2007) or when consumer pressure increases (Rousset & Lepart 1999; García & Obeso 2003; Baraza et al. 2006). However, only a few studies have tried to clarify the relative importance of the two mechanisms of facilitation in the same community (e.g. Callaway 1992; Gómez-Aparicio et al. 2008; Torroba-Balmori et al. 2015).

Moreover, some research has shown that both the net outcome and the strength of the interactions may vary depending on other aspects, such as levels of extreme stress (Michalet et al. 2006), the abiotic stress factor involved (Maestre et al. 2009), the performance measure considered (Maestre et al. 2005), the potential benefactor or facilitator species (Maestre et al. 2009; Rolo et al. 2013), tolerance/non-tolerance of the potentially beneficiary species to stress (Maestre et al. 2009; Madrigal-González et al. 2014) or the life stage of the interacting organisms, among others (Bertness & Callaway 1994). Further studies on these aspects are therefore necessary.

In the Iberian Peninsula, there is a transition between sclerophyllous and deciduous oak species that has traditionally been explained by interspecific differences in drought/shade tolerance (Pigott & Pigott 1993; Niinemets & Valladares 2008). On the one hand, *Quercus ilex* subsp. *ballota* Samp (sclerophyll; hereafter *Q. ilex*) is a well-adapted ecotype for mediterranean sub-humid–semi-arid conditions characteristic of the centre and south of the Iberian Peninsula. On the other hand, *Q. pyrenaica* Willd (marcescent) is a typical tree species of the mediterranean–temperate transition in Iberian areas, which occasionally co-exists with *Q. ilex* although it is more moisture-demanding and prefers sub-humid and humid mediterranean conditions (Rivas-Martínez 1987). Also, in areas with more gentle relief, these forests have frequently been transformed through livestock farming, giving rise to savanna-like landscapes. A dominant shrub species in these systems is *Cytisus multiflorus* (L'Hér.) Sweet, whose role in the establishment of *Quercus* species is not well known (Madrigal-González et al. 2014). Therefore, an ideal system to simultaneously test the importance of abiotic/biotic mechanisms of facilitation for species with contrasted traits of tolerance to stress under a bioclimatic limit can be found in the Iberian Peninsula.

In this study, we assessed the role of native shrubs (*C. multiflorus*) in the early establishment (survival and growth) of the two contrasted *Quercus* species (*Q. ilex* and *Q. pyrenaica*) in areas with significant summer drought and very poor soils, which increased the level of abiotic stress, and coupled with high grazing pressure. Specifically, the study addresses the following questions: (1) is there any substantial positive effect of native shrubs on seedling survival and growth of two contrasted *Quercus* species under a bioclimatic limit in Mediterranean grazed areas; (2) is the facilitation effect of nurse shrubs mediated by protection against herbivores or abiotic factor improvement; and (3) are there different patterns between species for the studied parameters according to their different ecological requirements?

Methods

Study site and species description

The experiment was conducted in northwestern of Salamanca, Spain (41°13′N, 6°24′W; 740–750 m a.s.l.). The climate is on the border between dry and sub-humid mediterranean (Luis Calabuig & Monserrat 1978), with 550–650 mm annual precipitation and annual mean temperature of 12.4 °C (1951–2013 means from Villarmuerto meteorological station; Fig. 1). Rainfall is not distributed evenly throughout the year, with a very pronounced summer drought, and varies greatly between years. Precipitation of the study years differed. Precipitation from Sept 2010 to May 2011 reached 528.6 mm (55.5 mm in Jun–Jul–Aug), whereas from Sept 2011 to May 2012 only 288.4 mm were recorded (17.4 mm in June–July–August). Soils are mainly dystric Cambisols (Soil Survey Staff 2010), i.e. oligotrophic, shallow, acid and with low water-holding capacity.

The study site is located in a transitional area between the domain of the *Q. ilex* to the east (drier) and the domain of *Q. pyrenaica* to the west (more humid) (Luis Calabuig & Monserrat 1978). The vegetation consists of low-density open stand formations of both *Quercus* species, with an herbaceous layer encroached by shrubs, the leguminous *C. multiflorus* being the dominant shrub species. These areas are traditionally used as pasture for cattle and sheep and shrubs are frequently burned in small patches. *C. multiflorus* regenerates well after disturbances such as burning and cutting and it can be classified as a facultative

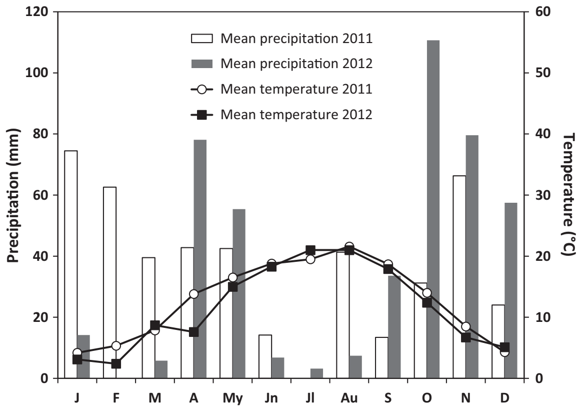

Fig. 1. Climate diagram for the study area during the years of experimentation (data from Villarmuerto meteorological station). Monthly precipitation during 2011 (open bars) and 2012 (solid bars), and monthly means temperature during 2011 (open circles) and 2012 (solid squares).

resprouter (Fernández-Santos et al. 1999; Paula et al. 2009). In recent years, the livestock load has increased from one cow 6 ha^{-1} (traditional load) to one cow 1 ha^{-1} (J. M. Gómez-Cuadrado, pers comm).

Experimental design and data collection

To evaluate the influence of nurse shrubs and herbivory on *Quercus* seedlings, a field experiment was conducted considering four treatments: (1) fenced open areas with no shrub cover, OF; (2) fenced areas under shrubs, SF; (3) non-fenced open areas, ON; (4) non-fenced areas under shrubs, SN. Five replicates of this experimental design were allocated randomly, at least 300 m apart from each other. Thus, five replicates (plots) of each treatment were available.

Large *C. multiflorus* shrubs (>1.5 m of crown diameter and around 15 yr old) were selected as nurse plants. The enclosures (3.5 m × 3.5 m) were made with mobile construction fences (2-m high × 3.5-m long, mesh holes: 12-cm wide × 22-cm long) fixed to concrete bases to prevent large herbivores from grazing. Non-fenced plots of the same dimensions were also established.

In each plot, ten tagged seedlings (1 yr old) of each target *Quercus* species were planted in March 2011 (i.e. a total of 200 seedlings of each *Quercus* species). Seedlings (Salamanca-Sayago provenance) were provided from the central nursery of the 'Junta de Castilla y León'. They were placed at a distance of 30 cm from each other, in one row all around the plot when they were planted in open areas, and in one row around the shrub basal stem 20–40 cm far from it when they were planted under shrubs. A small auger (diameter = 5 cm, depth = 20 cm) was used for planting to minimize damage to the shrub roots and soil structure disturbance. Soil was placed back in the holes and firmly pressed down around the root collar of the seedlings.

Seedling survival and growth were checked every 15 days during the two-first months after planting (Mar 2011), then monthly to the end of the first dry season (Oct 2011) and, afterwards, in spring and autumn to complete 2 yr (Apr 2013). The first survival evaluation (1 month after plantation, i.e. 1 Apr 2011) was considered as informative of post-plantation survival success (99% of seedlings survived). Survival was corrected through time for those apparently dead seedlings that resprouted in the following surveys; the majority of apparently dead plants were *Q. pyrenaica* seedlings (72%). Seedling height was measured from the root collar to the furthest living tissue at the time of planting and at each sampling date. Seedling growth was then estimated as the increase in seedling height (cm) through time.

In each plot, one composite soil sample was also taken. It consisted of five soil subsamples taken up to a depth of 15–20 cm, subsequently mixed and analysed for texture and chemical properties.

Soil analyses

Soil samples were air-dried, sieved (≤2 mm) and soil texture (sand, silt and clay content) analysed using the pipette method, after organic matter elimination with H_2O_2 and dispersion with sodium hexametaphosphate (Loveland & Whalley 1991). Soil pH was measured potentiometrically in a 1:1 soil:water suspension, using a CRISON digit, micropH 2001 instrument. Total organic matter was determined with k-dichromate oxidation, and total organic N using the Kjeldhal method (Bremner & Mulvaney 1982). Available Ca, Mg and K were extracted with ammonium acetate 1 M and determined by atomic absorption spectrophotometry (AAS). Finally, available P was determined using the Bray I method, modified from Bray & Kurtz (1945).

Data analyses

The effect of shrub cover on the physical and chemical soil properties was analysed using Student *t*-tests, once assumptions of normality (Shapiro-Wilk's test) and homoscedasticity (Levene's test) were met ($P < 0.05$). Seedling survival from planting through the following 2 yr was analysed with a Kaplan–Meier (K–M) method; when significant differences in global analysis were detected, paired comparisons with a log-rank test were made to find if the curves were parallel.

Survival and growth rates at particular times were compared with three-way ANOVA, factor 1 being: Shrub (levels: with-S, without-O), factor 2: Fence (with-F, without-N) and factor 3: Species (*Q. pyrenaica*, *Q. ilex*). Since the assumptions of normality and homoscedasticity were not met, data were transformed as follows: log $(x + 1)$ for survival rates and $\sqrt{x}$ for growth. When the ANOVA was significant, pair-wise comparisons of means were calculated with the Tukey's or Bonferroni test, depending on the equality in the number of replicates, i.e. Tukey test for survival rates and Bonferroni test for growth rates.

The Kaplan–Meier (K–M) method was performed with STATISTICA 8 (StatSoft, Tulsa, OK, US), whereas SPSS 20.0 was used for other analyses.

Results

Soil characterization

As a whole, the soils (Table 1) were very sandy (79.07 ± 0.38), with a very low percentage clay (3.87 ± 0.27), acid (5.18 ± 0.05) and very poor nutrient availability (Ca = 16.28 ± 2.47; Mg = 18.80 ± 3.16; K = 45.68 ± 5.29). The *C. multiflorus* canopy significantly enhanced the amount of organic matter (Shrub: 2.89 ± 0.28; Non-Shrub: 2.07 ± 0.14; $t = 2.46$, $P = 0.024$), but significantly reduced pH ($t = -6.06$, $P < 0.001$), K ($t = -3.87$, $P < 0.001$) and probably Mg ($t = -1.81$, $P = 0.086$).

Seedling survival

The Kaplan-Meier (K–M) survival curves for the whole study period showed significant differences between treatments for *Q. pyrenaica* (Fig. 2a) and *Q. ilex* (Fig. 2b) seedlings. Global analysis detected highly significant differences for both target species (log-rank test extended to various categories; *Q. pyrenaica:* $\chi^2 = 64.9$, $df = 3$, $P < 0.0001$; *Q. ilex*: $\chi^2 = 61.6$, $df = 3$, $P < 0.0001$). Pair-wise comparison with the log-rank test provided statistical differences between plots with and without shrub cover for *Q. pyrenaica* (SF-ON: 6.50, $P < 0.0001$; SF-OF: 5.44, $P < 0.0001$; SN-ON: 5.59, $P < 0.0001$; SN-OF: 4.48, $P < 0.0001$) and for *Q. ilex* (SF-ON: 4.65, $P < 0.0001$; SF-OF: 5.20, $P < 0.0001$; SN-ON: 4.33, $P < 0.0001$; SN-OF: 4.93, $P < 0.0001$). However, there were no differences between fenced and non-fenced areas for the two species (*Q. pyrenaica* SF-SN: 1.09, $P = 0.28$; OF-ON: 1.67, $P = 0.10$. *Q. ilex* SF-SN: -0.01, $P = 0.99$; OF-ON: 0.51, $P = 0.61$). In open areas, there was a sharp fall in seedling survival of both species after 60 days from planting (1 Jun) when the dry period had just begun (Fig. 1). Under the shrub canopy, the sharpest decrease in seedling survival occurred 1 (in SN) or 2 (in SF) months later than in the open areas for *Q. pyrenaica*, 1 month for *Q. ilex*. The total survival rate obtained after the first dry season was low for *Q. pyrenaica* (29.5%) and for *Q. ilex* (20.8%), but the values reached under the shrub canopy were higher than in open areas. Subsequently, there was hardly any mortality of seedlings, no cold period effect was seen in either of the 2 yr and a small decline in survival was found during the second dry season, higher for *Q. ilex* (from 20.3% to 16.3%) than for *Q. pyrenaica* (from 29.5% to 27.3%). The total survival rate obtained after 2 yr was 27% for *Q. pyrenaica* and 15.8% for *Q. ilex*.

The three-way ANOVA comparing survival rates after the first (A) and second (B) dry seasons found a significant effect of shrubs (A: $F = 11.57$, $P < 0.002$; B: $F = 19.02$, $P < 0.001$), but not of the fence (A: $F = 0.60$, $P = 0.44$; B: $F = 0.03$; $P = 0.87$) or *Quercus* species (A: $F = 0.001$, $P = 0.98$; B: $F = 0.39$, $P = 0.54$). Although significant interactions were not detected, the pair-wise comparisons were made because notable differences between mean values were observed. After the first dry period (Fig. 3a), *Q. pyrenaica* survival was significantly higher under the shrub canopy (SF: 58%, SN: 46%) than in open areas (ON: 2%, OF: 12%). There were no significant differences for *Q. ilex*, although survival was also higher under the shrub canopy. When comparing the two species, no statistical differences were found for any treatment. However, survival under the shrub canopy was higher in *Q. pyrenaica* than in *Q. ilex*, whereas in open non-fenced areas the trend was opposite and in open fenced areas survival rates were similar for both species. After the second year (Fig. 3b), some changes were detected with regard to the previous period. *Q. pyrenaica* survival was higher than *Q. ilex* in the fenced plots with shrub cover, (55% vs 24%, $P = 0.06$ probably significant differences) and *Q. pyrenaica* survival was significantly higher under shrub cover without a fence (SN: 44%) than in open fenced areas (OF: 8%).

Table 1. Results of soil physico-chemical analyses. The asterisks indicate significant differences ($P < 0.05$), and black dots probable significant differences ($P < 0.10$) between soils under shrub cover ($n = 10$) and in open areas with no shrub cover ($n = 10$).

Variable	Mean (±SE)		
	Shrub	No-Shrub	
Sand (%)	79.16 (±0.71)	79.07 (±0.38)	
Silt (%)	16.78 (±0.72)	17.09 (±0.54)	
Clay (%)	4.06 (±0.34)	3.87 (±0.27)	
pH	4.70 (±0.06)	5.18 (±0.05)	*
Available Ca ($mg \cdot kg^{-1}$)	14.36 (±2.12)	16.28 (±2.47)	
Available Mg ($mg \cdot kg^{-1}$)	12.35 (±1.41)	18.80 (±3.16)	•
Available K ($mg \cdot kg^{-1}$)	25.38 (±2.17)	45.68 (±5.29)	*
Organic matter (%)	2.89 (±0.28)	2.07 (±0.14)	*
Total N (%)	0.18 (±0.05)	0.29 (±0.10)	
C/N ratio	12.87 (±1.13)	9.85 (±1.74)	
Available P ($mg \cdot kg^{-1}$)	77.64 (±10.42)	66.11 (±7.75)	

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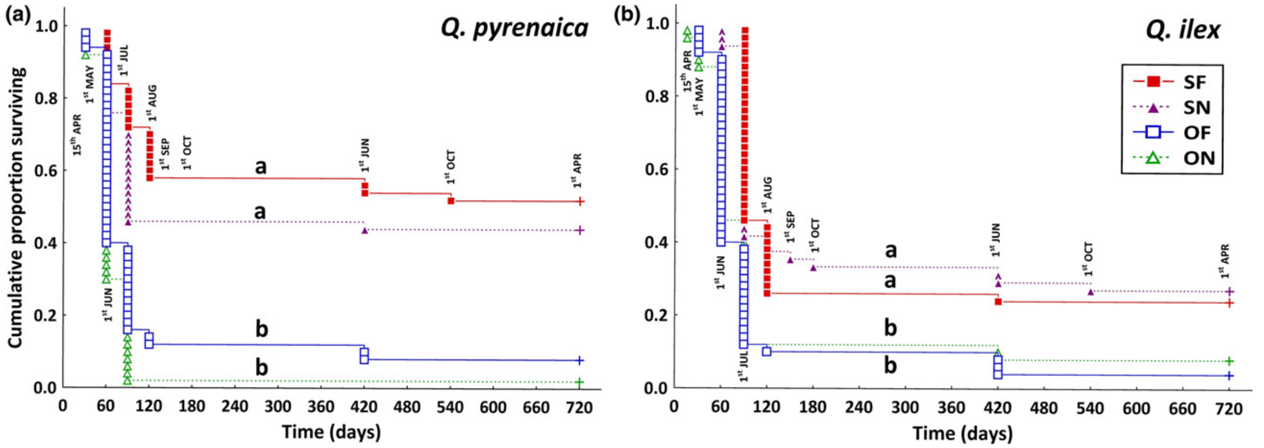

Fig. 2. Kaplan-Meier survival curves obtained for seedlings of each species in each treatment for the whole studied period. Different letters indicate statistically different groups according to the log-rank test ($P < 0.05$). SF, fenced areas under shrubs; SN, no fence areas under shrubs; OF, fenced open areas; ON, no fence open areas. [Color figure can be viewed at wileyonlinelibrary.com]

Seedling growth

Seedling growth from planting to the end of the first growing period (late Jun) was low: mean values of 4.4 cm for *Q. pyrenaica* and 2.0 cm for *Q. ilex*. The three-way ANOVA found a significant effect of shrubs ($F = 89.54$, $P < 0.001$), fence ($F = 37.02$, $P < 0.001$) and *Quercus* species ($F = 71.45$, $P < 0.001$), and a significant triple interaction ($F = 5.62$, $P = 0.02$) on seedling growth. *Q. pyrenaica* growth was significantly higher under the shrub canopy (SF: 6.5 cm, SN: 4.8 cm) than in open areas (OF: 3.8 cm, ON: 1.5 cm), and in fenced areas than in no-fence areas (Fig. 4A). *Q. ilex* growth was also significantly higher under the shrub canopy (SF: 3.7 cm, SN: 2.1 cm) than in open areas (OF: 1.3 cm, ON: 0.7 cm), but the fence only had a significant effect in plots with shrub cover (SF > SN), and not in open plots (OF, ON). When comparing the two species, *Q. pyrenaica* seedlings grew significantly more than *Q. ilex* in all treatments except in open no-fenced areas.

During the following months until the start of the second spring and throughout the cold period, seedlings hardly grew. Therefore, mean growth until early autumn was 0.6 cm and only occurred in 75% of seedlings.

The three-way ANOVA performed to analyse seedling growth from planting to the end of the second significant growing period (late Jun in the next year) showed a

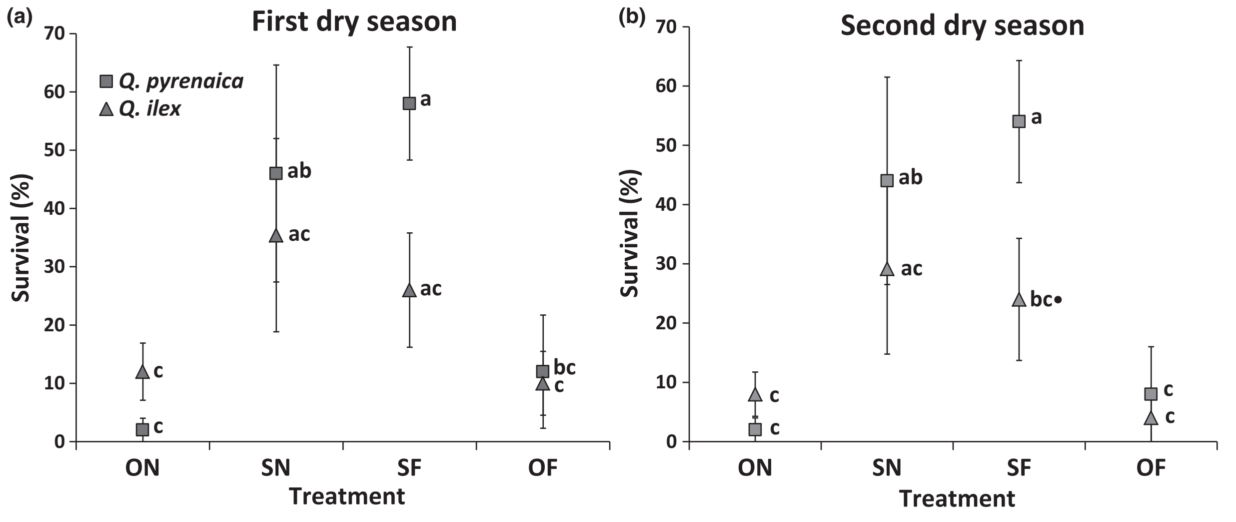

Fig. 3. Survival rates obtained for each species in the different treatments (mean ± SE). (**a**) After the first dry season; (**b**) after the second dry season. Different letters indicate significant pair-wise comparisons with Tukey's test ($P < 0.05$), except when letters have a black dot (•), which indicates probable significant differences ($P < 0.10$) between species in SF treatment in (**b**). Abbreviations as in Fig. 2.

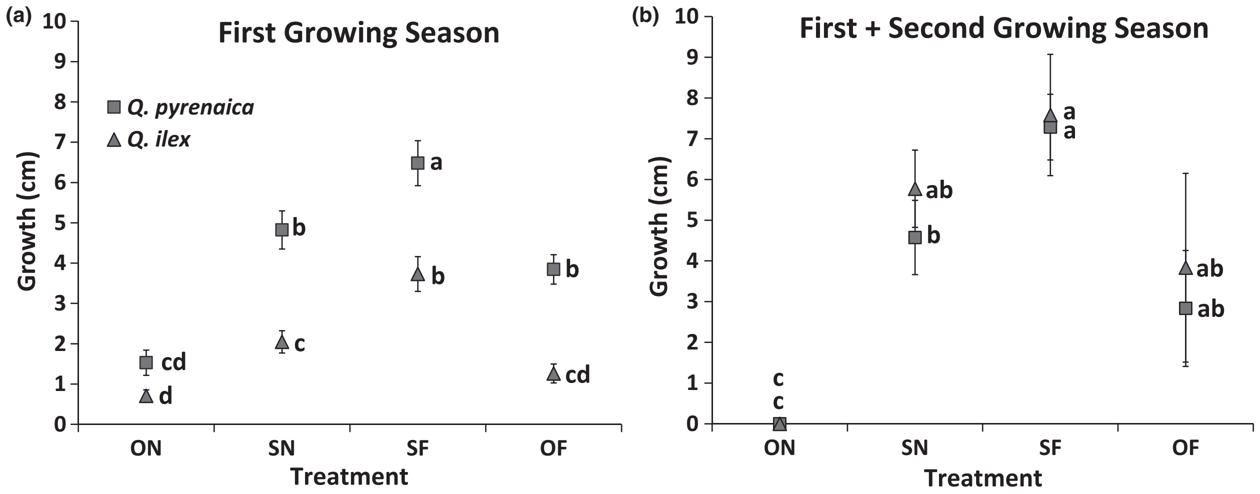

Fig. 4. Seedling growth (cm) for each species in the different treatments (mean ± SE). (**a**) First growing season; (**b**) first + second growing seasons. Different letters indicate significant pair-wise comparisons with Bonferroni's test ($P < 0.05$) after a three-way ANOVA (factors: species, shrub and fence). Abbreviations as in Fig. 2.

significant effect of shrubs ($F = 14.18$, $P < 0.001$) and fence ($F = 5.20$, $P = 0.03$) but not of *Quercus* species ($F = 0.14$, $P = 0.71$) or any interaction. However, the pair-wise comparisons (Fig. 4b) showed significantly higher seedling growth under shrub cover than in open areas for both species in the no-fenced plots (SN-ON), and was not significant in the fenced plots (SF-OF). The fence had a significant effect for both species in open areas (OF-ON), but only for *Q. pyrenaica* under shrub cover (SF-SN). Moreover, similar seedling growth was found for both species after the two growing periods, where for any differences detected with or without the shrub or with or without a fence, the highest mean values were on SF plots (*Q. pyrenaica*: 7.3 cm, *Q. ilex*: 7.6 cm; Fig. 4b).

Discussion

Seedling survival

Cytisus multiflorus facilitated *Q. pyrenaica and Q. ilex* seedling survival in comparison with open areas. The positive effect of other leguminous shrub species on *Quercus* seedling survival has also been described recently: *Retama sphaerocarpa* (Rolo et al. 2013), *Genista hirsuta* (Smit et al. 2007, 2008), *G. florida* and *Cytisus scoparius* (Torroba-Balmori et al. 2015). In the study area, regardless of species and treatment, *Quercus* seedling survival 2 yr after planting was low (21%), but similar to that found in reclaimed coal mine spoil in northern Spain (Torroba-Balmori et al. 2015) and lower than in other studies of reforestation, varying between 30% and 70% (Navarro-Cerrillo et al. 2005; González-Rodríguez et al. 2011; Núñez-Valero 2013; Rolo et al. 2013).

The positive effect of *Cytisus* on seedling survival may be due to amelioration of abiotic conditions and/or protection against large herbivores, as mentioned for other nurse species (García & Obeso 2003). Here, the highest mortality regardless of treatment occurred during the summer drought, but whereas in open areas the most marked decrease in seedling survival occurred as soon as the dry season started, under the shrub canopy the fall in seedling survival was less marked and later, when the dry season was more advanced. Moreover, in areas with shrubs the enclosures did not significantly enhance seedling survival, and in open fenced areas survival was negligible and similar to that in open non-fenced areas. Thus, in open areas enclosures did not have a positive effect either. Therefore, the most limiting factor for seedling survival seems to be summer drought, and the most important effect of *C. multiflorus* is the amelioration of abiotic conditions.

In particular, we found a positive effect of shrub canopy on soil properties such as organic matter, which, in turn, contributed to an increase in soil water-holding capacity. This is very important because of the very low water-holding capacity of the soils at these sites (very sandy and with little clay), as well as very scarce precipitation in the summer months. However, *C. multiflorus* significantly reduced the amount of K, unlike other shrub species (Gómez-Aparicio et al. 2005; Smit et al. 2008) where the amount of K increased under their canopy, and this seems to increase plant resistance to drought (Bradbury & Malcolm 1977; Egilla et al. 2001). Moreover, *C. multiflorus* may also have had a significant effect on microclimatic conditions as described for other shrub species (Gómez-Aparicio et al. 2005, 2008; Smit et al. 2008; Muhamed et al. 2013).

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On the other hand, this study clearly showed that the main critical period for *Q. pyrenaica* and *Q. ilex* seedling survival is the first summer. Mortality was practically zero during the non-summer periods and the second dry season, particularly in the case of *Q. pyrenaica*, even though precipitation during the summer months (17.4 mm) was lower than in the previous year (55.5 mm). These results are consistent with the argument that the first dry season is the most critical period for seedling survival (Navarro-Cerrillo et al. 2005; Quero et al. 2006; Puerta-Piñeiro et al. 2007). Nevertheless, some longer-term studies have shown that *Q. ilex* seedlings continue to die (Núñez-Valero 2013; Rolo et al. 2013), hence longer-term monitoring is needed to check whether there are more critical periods.

It is important to mention that to correctly assess *Q. pyrenaica* seedling survival after the dry season it is recommended to wait until the following spring as some apparently dead individuals resprout later, as mentioned previously (González-Rodríguez et al. 2011; Torroba-Balmori et al. 2015). This is probably because *Q. pyrenaica* seedlings and saplings represent a large investment of resources in development of the root system (Silla & Escudero 2006), hence their leaves are particularly prone to wither early, as has been demonstrated for other deciduous *Quercus* species (Silla & Escudero 2004).

The positive effect of *C. multiflorus* on seedling survival was more pronounced for *Q. pyrenaica* seedlings than for *Q. ilex* seedlings, particularly in the fenced areas, and this trend became significantly more apparent throughout the second year. These results support the idea that while positive interactions are hypothesized to be relevant for the persistence of mesophytic species at the margins of their distribution (Choler et al. 2001; Castro et al. 2006; Wang et al. 2008; Madrigal-González et al. 2012), competition would limit stress-tolerant species in the same areas that define bioclimatic transitions (Lookingbill & Zavala 2000), as mentioned by Madrigal-González et al. (2014). At our site, the shrub effect on *Q. ilex* seedling survival is not negative but less positive than on *Q. pyrenaica* seedling survival. Also, shrubs in our site are large and constitute old communities, so that our results agree with those of Madrigal-González et al. (2014), who found that old shrub formations favour the regeneration of *Q. pyrenaica* against *Q. ilex*, regardless of the identity of the shrub species. The mechanisms involved have not been analysed, but it has often been indicated that the different morphological characteristics of deciduous species make them more susceptible to water shortage than sclerophyllous species (Acheral & Rambal 1992; Costa et al. 1997; Fotelli et al. 2000). Therefore, in xeric environments, shade provided by shrub cover can improve conditions for *Q. pyrenaica* via a dual effect: light interception preventing photoinhibition (Gómez-Aparicio et al. 2006) and a reduction in transpiration demand and plant wilting during the dry season (Gómez-Aparicio et al. 2005).

Comparing the two species in open areas in general shows that protection against large herbivores has no clear positive effect on seedling survival of either species, but in any case slightly favours *Q. pyrenaica* over *Q. ilex*; in open fenced areas survival was similar for both species whereas in open non-fenced areas survival was lower for *Q. pyrenaica* seedlings. These findings confirm that in Mediterranean forests herbivores exert more pressure on deciduous species (*Q. pyrenaica* in this study) than on evergreens (*Q. ilex*), which are better defended and much less attractive to herbivores (Cuartas & García-González 1992).

Seedling growth

Quercus pyrenaica and *Q. ilex* seedling growth was very low in these Mediterranean areas, in contrast to values reported by other authors (Núñez-Valero 2013; Torroba-Balmori et al. 2015). For both species, seedling growth takes place in spring and is practically non-existent in summer. In open areas, without shrubs, no growth was recorded 2 yr after planting. Usually, *Quercus* species show little growth, especially when conditions of the first dry season are stressful (Navarro-Cerrillo et al. 2005). However, growth values reached in this study were extremely low, probably due to soils with low fertility, pH and water-holding capacity.

In these poor soils *C. multiflorus* seems to play a facilitator role in *Quercus* seedling growth, especially in the fenced areas, providing a favourable microenvironment for seedling growth, as shown for other shrub species (Núñez-Valero 2013; Torroba-Balmori et al. 2015). Nevertheless, we did not find a significant effect of *C. multiflorus* on soil nutrient content, contrary Gómez-Aparicio et al. (2005) under another shrub species. We even found significantly lower values of Mg and K, and a decrease in pH under the *C. multiflorus* canopy. Thus, the increase in height of seedlings might be due to an improvement in water availability, as a result of changes in soil properties (organic matter) and, probably, in the microenvironment. Previous studies showed that shrubs introduced changes in microclimatic conditions resulting in a positive effect on plant growth as well as on germination and survival (e.g. López et al. 2003).

However, the positive effect of the shrub on growth was not the same for both *Quercus* species and growth periods. The positive shrub effect was more important on *Q. pyrenaica* in the first growing period and on *Q. ilex* in the second, while 2 yr after planting no difference in shrub effect on growth of either *Quercus* species was detected. The lower level of growth in *Q. pyrenaica* under shrub cover in the first year in comparison with the second was also observed in reclaimed mined sites (Torroba-Balmori et al. 2015). The reason could be that *Q. pyrenaica* seedlings invest more resources in developing the root system (Silla & Escudero 2006), or in growing in other aerial dimensions after the first year, or that the ecophysiological advantages for oaks of environments under shrub cover, mentioned above in the survival section, are not clear when evaluating growth in poor soil environments. In fact, some ecophysiological studies carried out in the same region showed that differences in assimilation rate per unit area between *Quercus* species disappear at the seedling stage (Mediavilla & Escudero 2004).

Fence protection against large herbivores had a positive effect on *Quercus* seedling growth, at least during the first 2 yr after planting. Nevertheless, the effect of the fence was more important for *Q. pyrenaica* because higher growth was detected both with and without the shrub and in the 2 yr, whereas for *Q. ilex* differences were not always significant. In the non-fenced open areas, no growth was recorded 2 yr after planting, suggesting that consumption by herbivores was at least similar to the height increment (growth) of seedlings. Nevertheless, we have to consider that underground growth was not quantified in this study.

Conclusions

We conclude that in Mediterranean grazed areas affected by important summer drought and very sandy soils, shrubby plants of *C. multiflorus* have a clear facilitative effect on seedlings of ecologically contrasted *Quercus* species; this positive effect is particularly important during the first dry period. In these conditions, *C. multiflorus* appears to play a more important role in modifying abiotic factors than in protection against large herbivores; nevertheless, with regard to edaphic characteristics, a positive effect was only detected on organic matter, and therefore on soil water retention capacity. The facilitative effect was found for both marcescent and sclerophyllous oak seedlings, but to a different degree depending on the species considered and the variable measured (survival or growth). In terms of survival, the marcescent species (*Q. pyrenaica*) was more favoured by shrubs than the sclerophyllous species (*Q. ilex*), and this effect was accentuated through time. However, in terms of growth, although *Q. pyrenaica* was initially more favoured by the shrub, after 2 yr the facilitator effect was similar for both marcescent and sclerophyllous oaks. Therefore, *C. multiflorus* can have a key role in the restoration of degraded oak ecosystems, especially for marcescent species, under a bioclimatic limit in Mediterranean grazed areas.

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