



## Article

# The Effects of Native Shrub, Fencing, and Acorn Size on the Emergence of Contrasting Co-Occurring Oak in Mediterranean Grazed Areas

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**Abstract:** Research Highlights: The regeneration of *Quercus* species is usually very difficult in many oak woodlands transformed by livestock farming. Some studies have reported that shrubs can facilitate regeneration. However, the strength of interaction may vary depending on, among other factors, the shrub species and the stress tolerance of the oak species. Moreover, further studies are necessary to clarify the relative importance of the two facilitation mechanisms in the same community. Background and Objectives: *Cytisus multiflorus* (L'Her.) Sweet is a predominant shrub species in the Mediterranean grazed open-oak-woodlands found in the central west of the Iberian Peninsula (bioclimatic limit) and is present with *Quercus pyrenaica* Willd and *Quercus ilex* subsp. *ballota* Samp trees. Thus, we assessed the effect of these native shrubs and acorn size, and the effect of excluding large herbivores, on the seedling emergence of two contrasting co-occurring *Quercus* species under a bioclimatic limit. Materials and Methods: A manipulative field experiment was carried out considering four treatments as a combination of shrubs (shrub/no-shrub) and fence (fenced/open) factors. A total of twenty plots, five replicates for each treatment were available. In each plot, 20 acorns were sown: 10 acorns (5 small and 5 large) for each *Quercus* species. Acorn emergence was recorded during the first four years following the sowing. Results: Seedling emergence took place mostly in the spring of the first year after sowing. The presence of shrub was the main significant factor and incremented the emergence of both *Q. ilex* and *Q. pyrenaica*. The effect of the fence depended on the *Quercus* species considered, improving only the emergence of *Q. pyrenaica*. A negative effect with the small acorns was detected but only for *Q. pyrenaica*. In all treatments, *Q. ilex* emerged more than *Q. pyrenaica*. Conclusions: *C. multiflorus* had a clear facilitative effect on the seedling emergence of *Q. ilex* and *Q. pyrenaica*, which was much greater than the physical effect that acorn size and excluding large herbivores had. As such, this native shrub may have a key role in oak regeneration in Mediterranean grazed areas. Furthermore, in these areas of contact between marcescent and sclerophyllous *Quercus* species, *Q. ilex* currently emerges more than *Q. pyrenaica*. This could be indicative of a shift towards more xeric climatic conditions, which could lead to a change in the dominant tree species in the future. However, this change could be modulated by the effects of native shrub and large herbivores.



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**Keywords:** shrub cover; *Quercus ilex*; *Quercus pyrenaica*; *Cytisus multiflorus*; emergence; abiotic stress; facilitation; herbivory; Mediterranean environment

## 1. Introduction

Many oak woodlands worldwide have been transformed by livestock farming, giving rise to savanna-like landscapes [1,2] with a predominant pasture matrix of isolated oak

trees and shrubs. These landscapes have a high biological diversity that is associated with a high structural diversity [3,4] and oak trees are essential for the maintenance of ecosystem services [5]. The largest example of this landscape in Europe is the pastureland in the southwest of the Iberian Peninsula, covering almost 3.5 million hectares [6].

However, these woodlands are suffering dieback of adult trees [7] and the regeneration of *Quercus* species is found to be generally very difficult [8]. Studies regarding the demographic structures of *Quercus* populations have confirmed the almost complete lack of juvenile classes. The insufficient regeneration of oak trees has been explained mainly as a consequence of deficient seed dispersal to suitable sites for seedling establishment [6,8]. Moreover, reforestation is difficult because of their low rates of survival and growth, especially in the first dry season [9]. Therefore, it is of great interest to identify effective “safe sites” for *Quercus* regeneration to maintain the stability of these systems.

In the last decades, several studies have concluded that shrubs could play a role in oak regeneration [3,10,11]. The positive interaction of these shrubs should be dominant over the competition in communities under high abiotic stress or high consumer pressure [12]. Temporary abandonment and subsequent encroachment by shrubs have been proposed as useful tools to improve tree growth [13]. Numerous empirical studies have reported that shrubs can facilitate regeneration by improving abiotic conditions [14], reducing water stress and positively influencing the emergence and growth [6], or by protecting against herbivores [15–17]. However, few studies have tried to clarify the relative importance of these two mechanisms of facilitation acting simultaneously in the same community [18–20]. Research has also shown that the strength of the interactions may vary depending on other aspects, such as the potential facilitating species [4,21,22], the stress tolerance of these species [22,23], the levels of extreme stress [24], the abiotic stress factor [22], or the performance measure considered [20,25]. Therefore, more studies on these aspects are necessary.

*Quercus ilex* subsp. *ballota* Samp (sclerophyll; hereafter *Q. ilex*) and *Q. pyrenaica* Willd (marcescent) are two species of *Quercus* frequent in the Iberian Peninsula and their distribution has traditionally been explained by interspecific differences in drought/shade tolerance [26,27]. *Q. ilex* is well adapted to the Mediterranean subhumid or semiarid conditions, whilst *Q. pyrenaica* is a typical tree species of the Mediterranean-temperate transition in Iberian areas, which occasionally coexists with *Q. ilex*, but it is more moisture-demanding and prefers subhumid and humid Mediterranean conditions [28]. Moreover, in areas with more gentle relief, these forests have frequently been transformed through livestock farming, giving rise to savanna-like landscapes. Therefore, an ideal system to simultaneously test the importance of abiotic/biotic facilitation mechanisms for species with contrasting traits of tolerance to stress under a bioclimatic limit can be found in the Iberian Peninsula. *Cytisus multiflorus* (L’Her.) Sweet is a dominant shrub species in these systems, whose role in the establishment of *Quercus* species is not very well known [23]. A positive effect of this shrub species on the initial survival and growth of *Quercus* seedlings was shown in a planting experiment [20]. However, its effect on the emergence from acorns is unknown (there are no previous studies). On the other hand, it may also be of interest to analyze whether the size of the acorns can influence the emergence. The size of the acorns varies widely both within the same species and among species of *Quercus* [29], but in general, the acorns mass of *Q. pyrenaica* is higher than that of *Q. ilex* [29,30]. The seed mass determines the nutrients that the seedling will have when it germinates and some studies have shown that large seed mass has certain advantages during the early stages of the seedling since it aids in the survival of situations with a low carbon gain, such as shade conditions [29]. Furthermore, large seeds can confer a competitive advantage because they allow access to limited resources, like water, nutrients, or light, and it will promote higher survival [31].

In this study a manipulative experiment was carried out in a Mediterranean grazed area with *Q. pyrenaica* and *Q. ilex* trees, in the domain of the *Q. pyrenaica* tree canopy, to assess the role of the native shrub (*Cytisus multiflorus*), the acorn size, and the exclusion of

large herbivore on the seedling emergence of two contrasting co-occurring *Quercus* species. We predicted that: 1—This native shrub may increase the emergence rates of *Quercus* species, but this effect may (a) vary with *Quercus* species; (b) be more pronounced than that of only excluding large herbivores; and (c) be more or less significant depending on the acorn size. 2—There are different patterns between *Quercus* species for the studied parameters under a bioclimatic limit in Mediterranean grazed areas.

## 2. Materials and Methods

### 2.1. Study Site and Species Description

The field experiment was located in the northwest of the Salamanca region, central west Spain (41°13' N, 6°24' W; 740–750 m a.s.l.). The climate is Mediterranean, with 550–650 mm of mean annual rainfall and 12.4 °C of mean annual temperature. Rainfall is not distributed evenly throughout the year with pronounced summer drought and great variation between years. The soils are very oligotrophic, acidic, and sandy, with low water holding capacity [20].

The study site was in a transitional area between the *Q. ilex* domain in the east (drier climate) and the *Q. pyrenaica* domain in the west (more humid climate) [32]. The vegetation consisted of low-density open stand formations of both *Quercus* species, trees over 60 years old mainly *Q. pyrenaica*, and a herbaceous layer encroached by shrubs, with the leguminous *Cytisus multiflorus* being the dominant shrub species. These areas are traditionally used as pasture for cattle and sheep under extensive livestock farming; currently, mainly cattle with a high livestock load. Shrubs are frequently burned in small patches and *C. multiflorus* regenerates well after disturbances and can be classified as a facultative resprouter [33,34]. There are also wild species that consume acorns, such as wild boars, jays, and several species of small rodents.

### 2.2. Experimental Design

A field experiment was carried out to analyze the influence of shrub and large herbivore exclusion on *Quercus* emergence, considering also the size of the acorns. In a Dehesa of 800 ha, five sites (300 m apart) were randomly selected. At each site, four treatments were tested: (1) shrub—fenced (SF), (2) shrub—open (SO), (3) no-shrub—fenced (NF), and (4) no-shrub—open (NO) (see Figure A1 in Appendix A). Then in each treatment one plot of acorns was sown containing 10 large and 10 small acorns of both *Q. ilex* and *Q. pyrenaica* (total 100 acorns per treatment).

Large *C. multiflorus* shrubs, more than 1.5 m of crown diameter and around 10–15 years old, were selected as nurse plants. Enclosures (3.5 m × 3.5 m) were mobile construction fences (2 m high × 3.5 m long, mesh hole: 12 cm width × 22 cm length) fixed to concrete bases to protect against large herbivores. Open plots had similar dimensions to enclosures.

In October–November 2010, *Q. pyrenaica* and *Q. ilex* acorns were collected from nearby forests and stored at 4 °C until sowed in December 2010. Non-viable acorns (empty or preyed upon by insects) were excluded by floating them in water [35]. The wet weights of acorns were respectively:  $3.28 \pm 0.07$  g and  $5.50 \pm 0.10$  g for small and large *Q. ilex* acorns, and  $5.19 \pm 0.14$  g and  $7.94 \pm 0.33$  g for small and large *Q. pyrenaica* acorns.

In each plot, 10 acorns for each *Quercus* species (5 small and 5 large ones) were sown in an area of 60 cm × 50 cm, 4 rows × 5 columns. Acorn size and species were systematically alternated to avoid the possible edge effect and also to favor the identification of the species in the early stages of the seedlings (see Figure A2 in Appendix A). Acorns were buried into the soil at 2–3 cm, and holes were systematically separated from each other by 8–10 cm, with the surface being protected against rodents with a labeled wire mesh (1.2 cm mesh width) fixed by nails [19,36].

Acorn emergence was recorded during the first four years following the sowing. Seedling emergence of each species was checked on 19 different dates, every fifteen days during the first six months after they began to emerge (in April) to the end of the first dry season (October) and, afterward, in spring and autumn to complete three more years.

### 2.3. Statistical Methods

A multifactorial analysis of variance (ANOVA) was done with the emergence values (percentage) obtained at the end of the period of study. The four factors whose effects we wanted to analyze were: factor 1: shrub (levels: shrub/no-shrub); factor 2: fence (fenced/open); factor 3: *Quercus* species (*Q. pyrenaica* and *Q. ilex*); and factor 4: size (acorn size: small/large). The site was added as a random factor. All three-way and four-way interactions were eliminated from the model to avoid overfitting.

For the emergence analysis through the time, a four-way repeated-measures ANOVA was done. Data until June of the second year were included. Only one more emergence was later recorded and the repeated dates with the same frequency of emergence could result in a mask of the significance of the results. All four-way and five-way interactions were eliminated from the model to avoid over-fitting.

Since the assumptions of normality (Shapiro–Wilk’s test) and homoscedasticity were not met, data were arcsine transformed. When the ANOVA was significant, pairwise comparisons of means were calculated with Tukey’s test. All analyses were performed with IBM SPSS Statistics 25.0 software (IBM Corp., Armonk, NY, USA).

## 3. Results

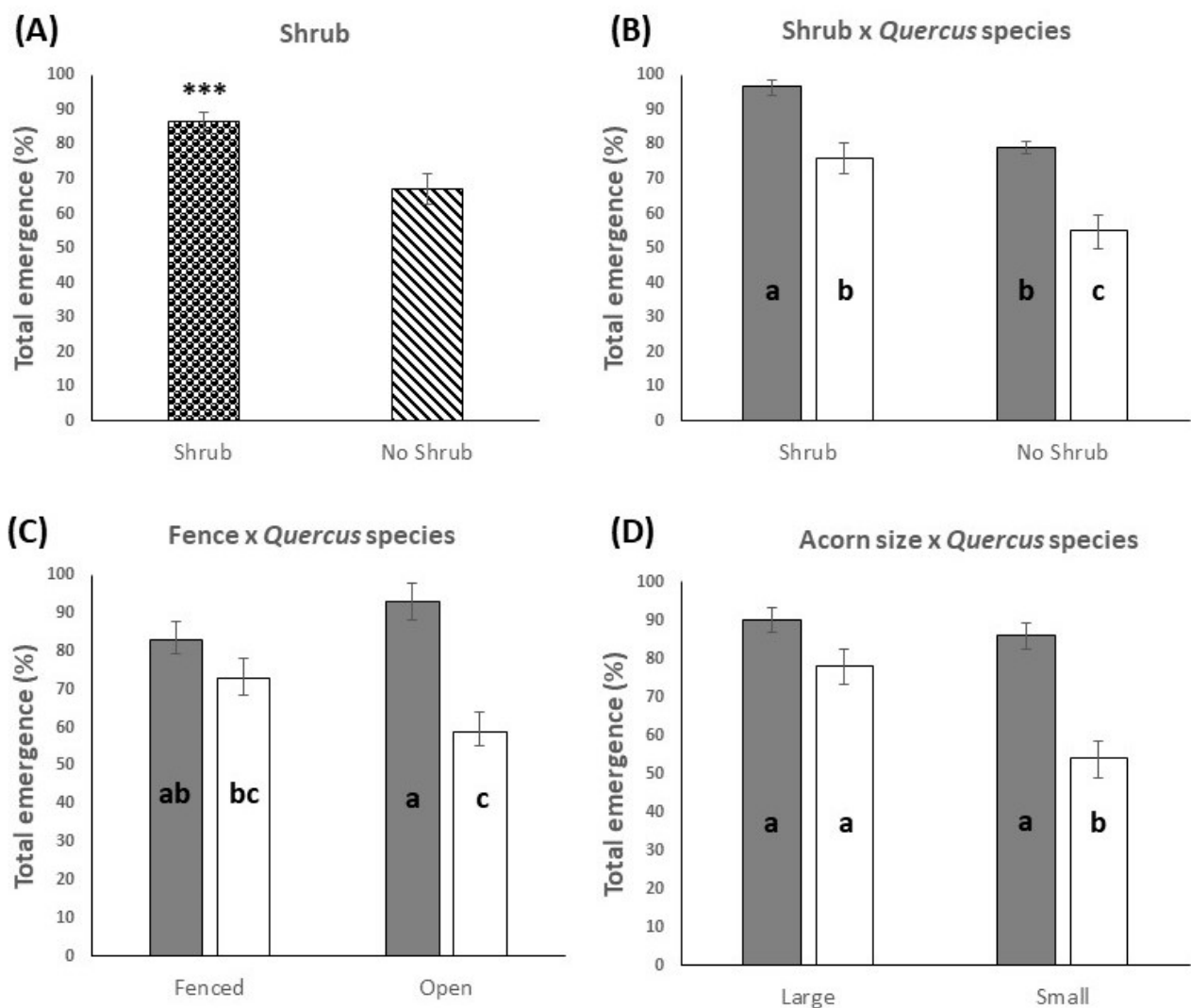
### 3.1. Emergence at the End of the Study Period

At the end of the study period, the overall percentage of emergence was 77%; the most contrasting average values were  $36\% \pm 13\%$  for small *Q. pyrenaica* acorns in the no-shrub—open (NO) treatment, and  $100\% \pm 0\%$  for large *Q. ilex* acorns in the shrub—open (SO) treatment.

The multifactorial ANOVA (Table 1) showed significant differences in emergence for the main effect of the shrub factor but without significant interaction with any other factors. The *Quercus* species factor and the interaction between fence and *Quercus* species factors were also significant. Therefore, the emergence was significantly higher in the Shrub treatment ( $87\% \pm 3\%$ ) than in the No-shrub treatment ( $67\% \pm 4\%$ ) (Figure 1A). This positive shrub effect was detected for both *Q. ilex* ( $97\% \pm 2\%$  vs.  $79\% \pm 5\%$ ) and *Q. pyrenaica* ( $76\% \pm 5\%$  vs.  $55\% \pm 6\%$ ) (Figure 1B). Nevertheless, the emergence increase in the shrub compared to the non-shrub treatment was somewhat higher for *Q. pyrenaica* than for *Q. ilex*; the increase ratio was 0.382 and 0.228, respectively. Moreover, *Q. ilex* emergence was greater than for *Q. pyrenaica* ( $88\% \pm 3\%$  vs.  $66\% \pm 4\%$ ) in both Shrub and No-shrub treatments (Figure 1B), and in open treatments ( $93\% \pm 4\%$  vs.  $58\% \pm 6\%$ ) (Figure 1C). There was no main effect of the fence factor on emergence when considering both *Quercus* species together ( $78\% \pm 4\%$  and  $75\% \pm 4\%$  in fenced and open treatments, respectively). However, when analyzing the fence  $\times$  *Quercus* species interaction, greater differences between fence and open treatments were observed for *Q. pyrenaica* ( $73\% \pm 5\%$  vs.  $58\% \pm 6\%$ ) than for *Q. ilex* ( $83\% \pm 5\%$  vs.  $93\% \pm 4\%$ ), with the balance being positive for the first species (Figure 1C). No main effect of the acorn size factor on emergence was detected but in interaction with *Quercus* species, being significant the differences between small and large *Q. pyrenaica* acorns ( $54\% \pm 5\%$  vs.  $78\% \pm 5\%$ ) but not for *Q. ilex* ( $86\% \pm 5\%$  vs.  $90\% \pm 3\%$ ) (Figure 1D). The interaction between acorn size and shrub showed that in open treatments the mean emergence values differed more between small and large acorns ( $59\% \pm 7\%$  vs.  $75\% \pm 5\%$ ) than in Shrub treatments ( $87\% \pm 4\%$  vs.  $86\% \pm 5\%$ ), although the differences were not significant.

**Table 1.** Results of the multifactorial ANOVA for emergence percentage data (arcsine transformed) obtained at the end of the study period.

Source	Sum of Squares	df	Mean Square	F	<i>p</i>
Shrub	1.681	1	1.681	20.016	<0.001
Fence	0.001	1	0.001	0.012	0.915
<i>Quercus</i> species	2.585	1	2.585	30.770	<0.001
Size	0.195	1	0.195	2.316	0.133
Site	0.691	4	0.173	2.056	0.097
Shrub × Fence	0.211	1	0.211	2.509	0.118
Shrub × <i>Quercus</i>	0	1	0	0.004	0.950
Shrub × Size	0.183	1	0.183	2.175	0.145
Fence × <i>Quercus</i>	0.724	1	0.724	8.621	0.005
Fence × Size	0.008	1	0.008	0.096	0.758
<i>Quercus</i> × Size	0.064	1	0.064	0.757	0.387
Error	5.460	65	0.084		

**Figure 1.** Emergence percentage (mean ± standard errorE) obtained in each treatment at the end of the study period, for both *Quercus* species together (A) and each *Quercus* species (B–D). Grey bars for *Q. ilex* and white bars for *Q. pyrenaica*. Results after Multifactorial ANOVA are shown. \*\*\* indicate highly significant differences (Tukey test;  $p < 0.001$ ). With letters results of pairwise comparisons between two-factor interactions; different letters indicate significant differences (Tukey test;  $p < 0.05$ ).



### 3.2. Emergence through the Time

Seedling emergence began 3.5 months after sowing and 98.4% of the total emergence taking place from April to October of the first-year. In the following 3 years, only 5 more seedlings emerged, four of them in the spring of the second year and one in the third year (1.3 and 0.3% of total emergence, respectively).

The repeated-measures ANOVA provided the following results. In the between-subject effects test (without analyzing the interaction with time), significant differences in emergence were only detected for the shrub ( $df = 1$ ,  $F = 19.471$ ,  $p < 0.001$ ) and *Quercus* species factors ( $df = 1$ ,  $F = 27.443$ ,  $p < 0.001$ ), and for the fence  $\times$  *Quercus* species interaction ( $df = 1$ ,  $F = 6.264$ ,  $p = 0.015$ ). These results, considering the whole temporal process, were like those obtained in the multifactorial ANOVA that used the accumulated emergence data obtained at the end of the study period, which is set out in the previous section (Table 1). In the tests of within-subject effects (Table 2), time was significant, and the two-way interactions of time with shrub, *Quercus* species, and acorn size. Additionally, two three-way interactions were significant: time  $\times$  shrub  $\times$  fence, and time  $\times$  shrub  $\times$  *Quercus* species.

**Table 2.** Results of the repeated measures analyses of accumulated emergence values (arcsine transformed) obtained through time.

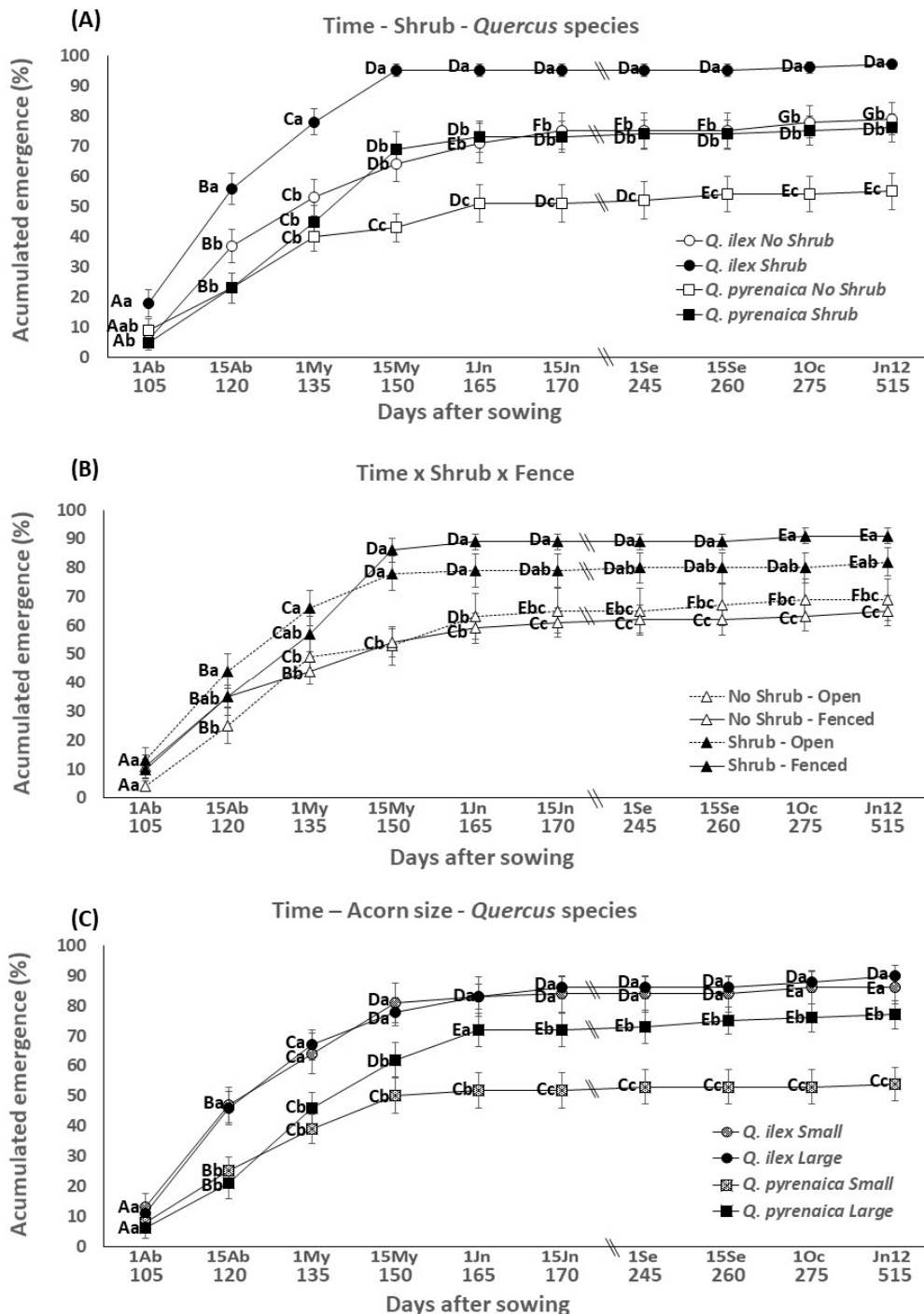
Source	Sum of Squares	df	Mean Square	F	p
Time	85.208	13	6.554	236.622	<0.001
Time $\times$ Shrub	2.007	13	0.154	5.574	<0.001
Time $\times$ Fence	0.342	13	0.026	0.95	0.5
Time $\times$ <i>Quercus</i>	1.214	13	0.093	3.37	<0.001
Time $\times$ Size	0.672	13	0.052	1.867	0.03
Time $\times$ Shrub $\times$ Fence	1.824	13	0.14	5.066	<0.001
Time $\times$ Shrub $\times$ <i>Quercus</i>	0.791	13	0.061	2.196	0.008
Time $\times$ Shrub $\times$ Size	0.188	13	0.014	0.523	0.912
Time $\times$ Fence $\times$ <i>Quercus</i>	0.529	13	0.041	1.469	0.123
Time $\times$ Fence $\times$ Size	0.054	13	0.004	0.149	1
Time $\times$ <i>Quercus</i> $\times$ Size	0.51	13	0.039	1.415	0.146
Error (Time)	24.847	897	0.028		

The three-way interaction time  $\times$  shrub  $\times$  *Quercus* species (Figure 2A) showed that for both *Quercus* species emergence took place entirely in the spring with only 1.65% in September of the first year. *Q. ilex* had greater and faster emergence in the shrub relative to the no-shrub treatment. *Q. ilex* emergence was greater than for *Q. pyrenaica* but in no-shrub treatment the significant differences were detected one month later than in the shrub treatments. *Q. pyrenaica* showed significantly greater emergence in shrub than non-shrub treatments by 15 May. For *Q. ilex* the shrub promoted faster emergence and earlier in the spring than for *Q. pyrenaica*.

The three-way interaction time  $\times$  shrub  $\times$  fence (Figure 2B) showed that 15 days after the start of the emergence, there were already significant differences between open treatments, with shrubs (SO: 44%  $\pm$  6.2%) and without shrubs (NO: 25%  $\pm$  6.3%), while no differences were detected between the fenced treatments (SF and NF) during the first month. From 15 May, significant differences appeared between shrub (SF: 86%  $\pm$  4.1% and SO: 78%  $\pm$  5.9%) and no-shrub treatments (NF: 54%  $\pm$  4.8% and NO: 53%  $\pm$  6.7%), with no significant fence effect. The emergence stabilized in July and since then, the greatest emergence was found in the SF treatment (91%  $\pm$  3%), significantly higher than in the no-shrub treatments (NF and NO), followed by the SO treatment (82%  $\pm$  5%) that significantly differed to NF, and finally, the no-shrub treatments (65%  $\pm$  5% NF and 69%  $\pm$  7% NO) that did not differ from each other.

The three-way interaction time  $\times$  size  $\times$  *Quercus* species (Figure 2C) showed that the acorn size did not have a significant effect on the emergence of *Q. ilex* over time but did for *Q. pyrenaica*. For *Q. ilex*, the emergence for large and small acorns was similar on each

date, and the accumulated emergence increased until 15 May and then stabilized. For *Q. pyrenaica*, the emergence was significantly greater for large acorns than for small ones from 1 June. Regardless of the acorn size, the emergence for *Q. ilex* was significantly higher than for *Q. pyrenaica* from mid-April.



**Figure 2.** Accumulated emergence values (mean ± standard error) for each *Quercus* species and treatment on different dates throughout the first year and a half after sowing. Results after repeated measures analyses are shown. With letters and numbers results of pairwise comparisons between three-factor interactions. Different capital letters indicate significant differences (Tukey test;  $p < 0.05$ ) between dates within the same *Quercus*-species (A,C) or treatments (B). Different lower-letters indicate significant differences (Tukey test;  $p < 0.05$ ) between *Quercus*-species (A,C) or treatments (B) at the same date.

#### 4. Discussion

*Cytisus multiflorus* facilitated the emergence of *Quercus ilex* and *Q. pyrenaica* in comparison with non-shrub areas. The positive effect of this shrub species on the acorn emergence of *Quercus* species has not been published before but on the survival and growth of seedlings of both target *Quercus* species [20]. The positive effect of other leguminous shrub species on *Quercus* emergence has also been described in the last decades: *Genista hirsuta* Vahl [37], *Retama sphaerocarpa* (L.) Boiss. [4], *Genista florida* L., and *Cytisus scoparius* (L.) Link [19]. However, other interesting results are found when evaluating the effect of excluding large herbivores, the acorn size, and the species of *Quercus* considered.

Regardless of treatment, *Quercus* emergence in the study area was much higher than in other areas for *Q. ilex* [4,37] and for *Q. pyrenaica* [19]. However, similar emergence values were obtained for *Q. pyrenaica* in other studies that used mesh protection to prevent acorn predation by small mammals [36]. Mesh protection has proved to be very effective in our study area because the removal of acorns by small mammals was near 100% when mainly under shrub cover (unpublished data), while high emergence values were obtained using a mesh. Acorn predation by small mammals is a common problem for *Quercus* regeneration even if acorns are buried [38,39], and mesh protection was also effective in improving acorn emergence [18,19,36]. Here, emergence took place mainly during the first spring, and only a minimum percentage of acorns emerged later. It is well known that acorns are recalcitrant and their viability decreases because of dehydration when outdoors for some time [40]. As such, the emergence of *Quercus* acorns is conditioned by soil moisture [41]. In these Mediterranean areas, soil moisture decreased significantly during the dry summer period because precipitation is very scarce then and the soil is very sandy, with a very low water-holding capacity [20]. We also found that some acorns presented a delayed emergence, as found in other studies [19,36], which happens when germination occurs late due to the summer drought inhibiting their growth until the moisture conditions become optimal again [36].

When analyzing the effect of the different factors, the clearest result was the positive effect of the native shrub, *Cytisus multiflorus*, on the emergence of both *Quercus* species, which was higher from the beginning and faster in shrub areas. Other leguminous shrubs have also shown a facilitating effect on the emergence of *Q. pyrenaica* [19] or *Q. ilex* [4,39], or on the survival and growth of *Quercus* seedlings [4,19,20]. Our results are most likely due to the significant increase in organic matter and a slightly higher percentage of clay under the *C. multiflorus* canopy [20], which, in turn, contributed to an increase in soil water-holding capacity that is known to be very important for the germination and emergence of *Quercus* species [40,42,43]. As such, *C. multiflorus* improves the emergence of *Quercus* by preventing the drying out of the acorns, like other shrub species [39,44,45], which is very important in Mediterranean areas with a very low water-holding capacity in the soil. *C. multiflorus* may also have a significant effect by improving other abiotic (microclimatic) conditions, as described for other shrub species [18,39,46,47], or through protection against herbivores, as also mentioned for other species [15–17].

Regarding the use of the fence to exclude large herbivores, interesting interactions were found. The fence favored the emergence of *Q. pyrenaica* but not that of *Q. ilex* and reduced the differences in emergence between the two species of *Quercus*. On the other hand, the fence delays emergence differences detection between shrub and non-shrub areas compared to open areas, being higher the emergence under shrubs. This indicates that the shrub had initially (first month since the beginning of the emergence) a protective role against large herbivores. However, later (after the first month), the main effect of *C. multiflorus* was rather the improvement of abiotic conditions, since the fence no longer affects the emergence. Studies in the same area concluded that the main facilitating effect of *C. multiflorus* on *Q. ilex* and *Q. pyrenaica* seedling survival was the improvement of abiotic conditions, whereas the fence also affected seedling growth, mainly for *Q. pyrenaica* [20]. In other areas, no significant effect of the fence was detected on the emergence of *Q. pyrenaica* and *Q. petraea* seedlings [19]. Many studies show an important effect of rodents, jays,



and large herbivores on acorn consumption [8,38,48–52]. However, no evidence of acorn predation was found in our study as the protective meshes were not altered and no nearby rodent burrows were observed. The effect of herbivores in the study area is more likely to be caused by trampling, as domestic livestock are very abundant and tend to graze more in areas without shrub. We did not find studies on the effect of trampling on the comparative emergence of these two *Quercus* species, however, the trampling can compact the soil and alter its ability to absorb or retain moisture [53,54] or may also reduce tree transpiration and growth [55,56]. These soil modifications may affect the germination and emergence of *Q. pyrenaica* more than *Q. ilex*, as *Q. pyrenaica* is less tolerant to the dehydration of its acorns [57] and a water deficit in the development of seedlings [20]. Future studies to shed more light on this topic would be welcome.

By studying the effect of acorn size on emergence, different behaviors were shown in both species. Large acorns of *Q. pyrenaica* showed a higher emergence rate, while for *Q. ilex* no significant effect of acorn size on emergence was found. Other studies also find that acorn size can benefit the emergence of *Quercus* species [29], specifically *Q. pyrenaica* [36,58,59], whereas for *Q. ilex*, this does not always happen [60] and the relationship may only hold for some maternal plants [36,61]. On the other hand, the positive relationship between acorn size and emergence for *Q. pyrenaica* only occurs in open areas. It seems that a shrub provides more favorable conditions for the emergence of both species and, in such conditions, the acorn size loses importance. Some studies describe the effects of environmental factors on acorn emergence [62,63] and the positive effect of acorn size on the emergence and establishment in soils with low fertility or other adverse characteristics [64]. However, despite no major advantages of acorn size being observed in this study, especially for *Q. ilex*, it may be important for other aspects of *Quercus* establishment such as producing larger seedlings with longer roots, which increase the chances of surviving the summer drought [31,65–67], favoring the resprouting after herbivore action [68], or facilitating the establishment of seedlings in shaded environments, where acorns are frequently dispersed by jays and rodents [38,69].

By comparing the two *Quercus* species, the emergence was always higher for *Q. ilex* than for *Q. pyrenaica*, both with and without shrub. The emergence increase in shrub areas compared to non-shrub areas was somewhat higher for *Q. pyrenaica* than for *Q. ilex*. These results support the idea that positive interactions are relevant for the persistence of mesophytic species at the margins of their distribution areas [70–73]. On the other hand, in shrub areas, the emergence of *Q. ilex* was close to 100% and the process was faster, with there being the detection of differences with *Q. pyrenaica* earlier than in open areas. Therefore, even though shrubs in our study site were large and constituted old communities, our results regarding emergence do not support the idea that old shrub formations favor the regeneration of *Q. pyrenaica* against *Q. ilex*, as proposed by other authors [23]. However, in this study, only the emergence was assessed and other important aspects for the establishment of *Quercus* species should also be considered, such as the survival and growth of the seedlings. In another study in the same area, *C. multiflorus* favored the survival and early growth of *Q. pyrenaica* and *Q. ilex* but to a different degree depending on the variable measured [20]; the marcescent species survival was more favored than the sclerophyllous species, whereas the initial advantage of *Q. pyrenaica* in terms of growth was attenuated after 2 years.

Given our results, it seems that *Q. pyrenaica* has more problems with the emergence in the study area, the values are lower and emergence, in general, taking place later. In other sites, however, deciduous and marcescent species such as *Q. pyrenaica* emerged earlier than the evergreen ones like *Q. ilex* [36,58]. Even the emergence date appears to be a species-specific character [74], although this can be modified by the maternal source [36]. These differences obtained have surprised us even more since in the area the trees over 60 years old are *Q. pyrenaica*, with specimens that are even 200 years old, and only a few young trees of *Q. ilex* can be observed. Therefore, the clear advantage in the emergence of *Q. ilex* over *Q. pyrenaica* could be interpreted as a reflection of the change

towards more xeric conditions that has been taking place for some decades, and that will be even more accentuated in the center of the Iberian Peninsula [75], which, in the future, could lead to a change in the dominant tree species, from marcescent to sclerophyllous. However, although *Q. ilex* is more resistant to water deficit than *Q. pyrenaica* [76], in xeric environments, shrublands can improve the conditions for *Q. pyrenaica* by protecting it from sunlight and photoinhibition [18] and by reducing the demand for transpiration during the dry season [46], as was observed in this area when assessing the initial survival of seedlings [20]. Thus, if other phases of plant establishment, mainly survival and growth, are also considered, the change in the dominant tree species in our areas may be slower if there are shrubs. It would be of great interest to have further studies assess the process of interactions between *Quercus* species and shrublands in the medium and long term.

## 5. Conclusions

We conclude that in Mediterranean grazed areas, *C. multiflorus* has a clear facilitative effect on the seedlings' emergence of two ecologically contrasted *Quercus* species, *Q. ilex* and *Q. pyrenaica*. The presence of shrub has a much greater effect than the physical exclusion of large herbivores (fence); that is, initially it seems to play a protective role against large herbivores but, after the first month, *C. multiflorus* seems to play a more important role in modifying abiotic factors. Furthermore, the size of the acorn seems to be less important in the presence of shrub. Therefore, *C. multiflorus* can have a key role in oak regeneration in Mediterranean grazed areas.

On the other hand, in these Mediterranean grazed areas of contact between marcescent and sclerophyllous species of *Quercus*, with the domain of the *Q. pyrenaica* tree canopy, *Q. ilex* emergence is currently greater than for *Q. pyrenaica*. The emergence of *Q. pyrenaica* seems to be negatively affected if it is not protected against large herbivores and if the acorns are smaller, which is not the case for *Q. ilex*. This could be indicative of the shift towards more xeric climatic conditions that are taking place, which could lead to a change in the dominant tree species in the future, from marcescent to sclerophyllous, and this change could be more rapid because of pressure from large herbivores. On the other hand, the facilitative effect of *C. multiflorus* has been found for both oak species but, although it accelerates the process in *Q. ilex*, the increase ratio compared to the emergence outside is somewhat higher for *Q. pyrenaica*. Therefore, the change could be slower if there are shrubs. Therefore, further studies would be necessary to assess the process of interactions between *Quercus* species and the shrubland in the medium and long term.

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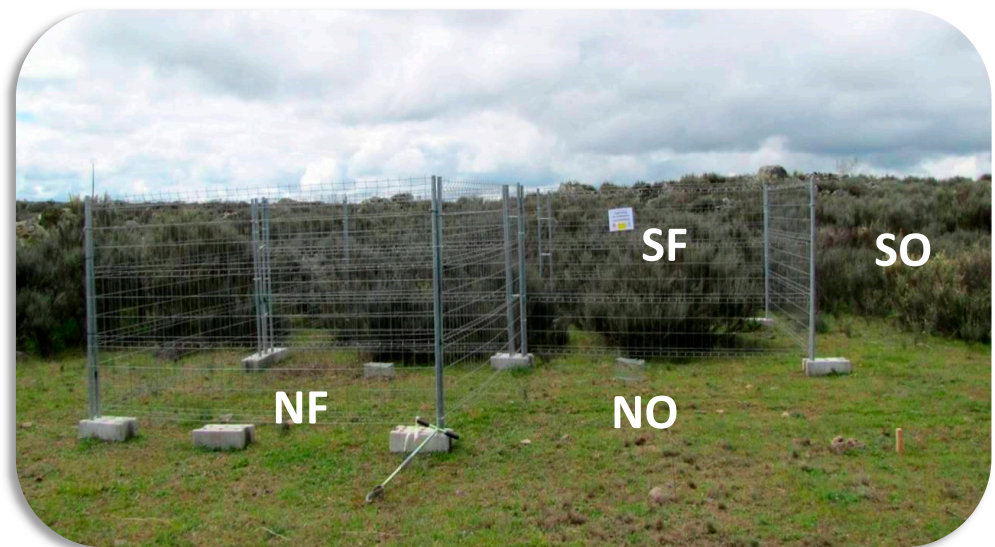
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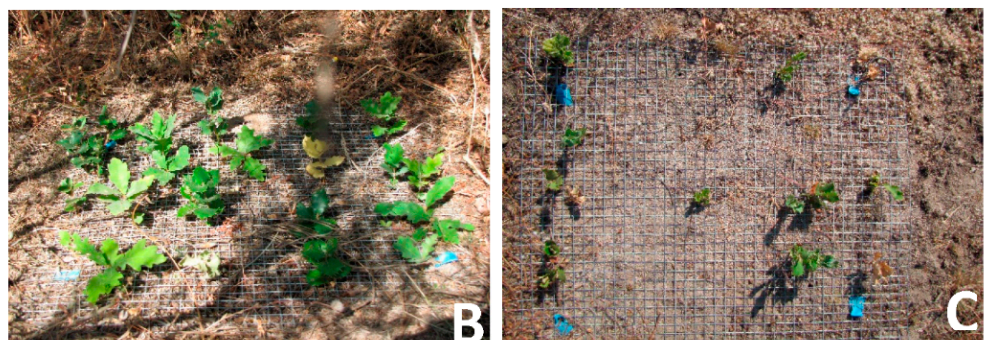
## Appendix A



**Figure A1.** Experimental design in the field. Spatial allocation of the four experimental plots, one for each treatment, considered in each selected area. Shrub—Fenced (SF), Shrub—Open (SO), No-shrub—Fenced (NF), and No-shrub—Open (NO).

**A**

Qi(l)	Qp(s)	Qi(l)	Qi(s)	Qp(l)
Qi(l)	Qp(s)	Qp(s)	Qi(s)	Qp(l)
Qi(s)	Qp(l)	Qi(s)	Qi(l)	Qp(s)
Qi(s)	Qp(l)	Qp(l)	Qi(l)	Qp(s)



**Figure A2.** Experimental sowing design carried out in each plot. (A)—Schematic of the basic experimental unit consisted of a 20-hole acorn seeding area in a disposition of 4 rows  $\times$  5 columns; five holes with large acorns (l), and five holes with small acorns (s) of each *Quercus* species. In the first and fourth columns, only seeds of *Quercus ilex* (Qi) were placed, in the second and fifth columns only acorns of *Q. pyrenaica* (Qp) were placed, and in the third column acorns of both *Quercus* species were placed. Acorn size and species were systematically alternated to avoid the possible edge effect and also to favor the *Quercus* species identification in the early seedlings stages. (B)—Seedlings emergence in the Shrub treatment. (C)—Seedlings emergence in the No-shrub treatment.



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