

Direct and indirect effects of tree canopy facilitation in the recruitment of Mediterranean oaks

Maria C. Caldeira^{1*}, Inés Ibáñez², Carla Nogueira¹, Miguel N. Bugalho³, Xavier Lecomte¹, Andreia Moreira¹ and João S. Pereira¹

¹CEF, ISA, University of Lisbon, Tapada da Ajuda, 1349-017 Lisbon, Portugal; ²School of Natural Resources and Environment, University of Michigan, 440 Church St., Ann Arbor, MI 48109-1041, USA; and ³CEABN, ISA, University of Lisbon, Tapada da Ajuda, 1349-017 Lisbon, Portugal

Summary

1. Tree recruitment in Mediterranean ecosystems is strongly limited at the seedling stage by drought. Increasing evidence shows the critical positive role of the canopy nurse effect on seedling survival which results from direct and indirect, positive and negative interactions between species.

2. Most studies, however, have only focused on the effects of tree canopy on water and light, ignoring other critical factors affecting seedling regeneration, such as canopy effects on high temperatures and the competing herb biomass.

3. Here, we evaluate how tree canopy cover and removal of herbs affect the survival and growth of seedlings of two dominant Mediterranean *Quercus* species during a 3-year study. We use an integrated model that combines several data sets to quantify and predict regeneration dynamics along environmental gradients of soil moisture, temperature and light.

4. Low soil moisture, increased soil temperature and herb biomass negatively affected seedling survival of both *Quercus* species. Seedling growth was positively associated with increasing soil moisture and light.

5. Although tree canopy cover directly facilitated seedling survival in both *Quercus* species, it also negatively affected herb biomass and thus indirectly facilitated the survival of *Quercus suber*, but not of *Quercus ilex* seedlings at low levels of soil moisture.

6. Overall, tree canopies increased seedling survival but not growth during the establishment phase, mainly by ameliorating the effects of low soil moisture and high temperatures. Tree canopy indirectly facilitated survival of *Q. suber* seedlings by negatively affecting the competing herb layer.

7. *Synthesis and applications.* To improve tree recruitment and conserve Mediterranean *Quercus* woodlands, the removal of herbs should be integrated into management plans for dry habitats. Interactions between abiotic and biotic factors may also effect the regeneration of these tree species. In particular, a healthy tree canopy will become important for providing conditions to facilitate seedling establishment if these habitats become drier and warmer, as predicted by some climate change scenarios.

Key-words: climate change, herb management, high temperatures, positive interactions, *Quercus ilex*, *Quercus suber*, tree recruitment

Introduction

Environmental conditions, including those mediated by plant–plant interactions, have large effects on tree recruitment, especially in semi-arid or arid ecosystems, such as the Mediterranean Basin (Castro *et al.* 2004; Gómez-Aparicio *et al.* 2005). In these ecosystems, plants are

exposed to low water availability and heat stress during summer (Pereira *et al.* 2006), limiting tree seedling survival (Marañón *et al.* 2004; Gómez-Aparicio *et al.* 2008). In the last decade, an increasing, but not unanimous, amount of experimental evidence suggested a critical role of plant–plant interactions in the survival and growth of tree seedlings in the region (e.g. Maestre & Cortina 2004; Gómez-Aparicio 2009). Understanding how the combination of environmental conditions affect the survival and

*Correspondence author. E-mail: mcaldeira@isa.utl.pt

growth of seedlings during the critical establishment phase is far from resolved (Valladares & Niinemets 2008) and is of high interest for restoration management practices and for modelling vegetation dynamics that include climate change scenarios (Ibáñez *et al.* 2007).

Plants directly modify the environment of other plants by competing for resources like water or light or by facilitative mechanisms such as amelioration of extreme temperatures or increasing resource availability (e.g. nutrients) (Callaway & Walker 1997; Brooker *et al.* 2008). In the Mediterranean ecosystems, the net facilitation effect on seedlings by adult trees or shrubs (canopy nurse effect) seems to play a key role in seedling recruitment (e.g. Castro *et al.* 2004; Gómez-Aparicio *et al.* 2005), and based on this, canopy nurse techniques are increasingly applied in restoration management plans (Padilla & Pugnaire 2009; Leiva, Mancilla-Leyton & Martin-Vicente 2013). Nevertheless, facilitation processes are complex as they depend on biotic (e.g. seedlings specific traits, seedling size) and abiotic conditions (soils, weather), some of which fluctuate from year to year (Holmgren, Scheffer & Huston 1997; Gómez-Aparicio *et al.* 2004; Holmgren *et al.* 2012). For example, competitive interactions may prevail over facilitation in particularly wet years (Tielbörger & Kadmon 2000; Holzapfel *et al.* 2006), or when tree seedlings are large (Liancourt, Callaway & Michalet 2005; Cuesta *et al.* 2010). Indirect interactions established between tree nurse canopies and herbs can affect seedling survival. Herb competitive capacity (Rey Benayas *et al.* 2002, 2005) may be reduced by the tree canopy (Cuesta *et al.* 2010; Prévosto *et al.* 2012), through competition for light and water, which can indirectly facilitate seedling survival.

In the Mediterranean Basin, evergreen *Quercus* woodlands occupy c. 2.5 million ha and have high conservation and socio-economic value (Bugalho *et al.* 2011). In the last few decades, these woodlands experienced a strong regeneration decline and a decrease in the density of mature trees, threatening its sustainability in many sites (Plieninger, Rolo & Moreno 2010; Bugalho *et al.* 2011). Most studies have addressed the effect of water and light on survival and growth of seedlings (e.g. Puerta-Piñero, Gómez & Valladares 2007; Urbieta *et al.* 2008), while few have focused on the effect of high temperatures and indirect interactions with the herb layer on the performance of seedlings under nurse canopies (e.g. Gómez-Aparicio *et al.* 2005; Cuesta *et al.* 2010; Holmgren *et al.* 2012). Accounting for the combined effect of these factors could be crucial as heat stress and herb competition for water can interact and exacerbate the effect of water and light stress (Chaves, Maroco & Pereira 2003; Rey Benayas *et al.* 2005), becoming critical variables influencing tree recruitment and thus compromising restoration management plans of evergreen *Quercus* woodlands.

In this study, we conducted a 3-year field experiment to assess direct and indirect environmental effects of canopy nurse trees on the recruitment dynamics of two co-existing evergreen drought and shade-tolerant *Quercus* species

(*Q. suber* L. and *Q. ilex* subsp. *rotundifolia* Lam.). We sowed pre-germinated acorns below the canopy and in open habitats, where we also manipulated the presence of the herb layer in both habitat types. We developed an integrated hierarchical Bayesian model to analyse seedling survival as a function of the environmental factors known to drive recruitment in these ecosystems. Specifically, we aimed to (i) understand and predict the role of abiotic factors, namely high temperatures and soil moisture, on survival and growth of the seedlings of both *Quercus* species under canopy nurse trees; and (ii) understand how direct and indirect biotic interactions affect seedling recruitment of these two species.

Materials and methods

We conducted this study at Tapada Real de Vila Viçosa, a 900-ha estate, in south-east Portugal (38°47'N, 7°22'W). Total precipitation, falling mainly in autumn and winter, during the 3 years of the experiment, was 401 mm (2004), 364 mm (2005) and 598 mm (2006) and mean annual temperatures ranged from 8 °C in winter (January) to 24 °C in summer (July). Precipitation and air temperature were collected from two weather stations (Estremoz – <http://snirh.pt>, and Mitra-www.cge.uevora.pt) with similar weather, located at 13 and 57 km from the study site. Soils are poorly developed haplic leptosols (WRB 2006) with dominant bedrock of schist. Plots were established in a mixed *Q. suber* L. (cork oak) and *Q. ilex* ssp. *rotundifolia* Lam. (holm oak) savannah-type ecosystem. The understorey was composed of annual herbaceous species that set seed and die in late spring with *Cistus ladanifer* L. shrubs in some areas.

EXPERIMENTAL DESIGN

Eighteen paired plots (2 × 4 m) were established in five pre-existing 625-m² fenced areas that excluded red deer *Cervus elaphus* L. and fallow deer *Dama dama* L. Ten plots were established randomly under the canopy of mature *Quercus* trees and eight in open grassland areas. Each plot was divided in two 2 × 2 m subplots. In one subplot, herb vegetation was removed by hand three times per year. Acorns of oak of both species were collected in the field and pre-germinated in the greenhouse. We used acorns of similar size (fresh mass) in the range of 3.5 g ± 0.9 for *Q. suber* and 2.8 g ± 0.8 for *Q. ilex* to avoid any potential effects of seed size in our experiment. When the radicles were c. 0.5 cm in length, acorns were buried 2 cm deep into the soil 30 cm apart. We sowed 36 acorns of each species in each subplot (totalling 1296 acorns per species). All seedlings were tagged. The experiment was conducted from November 2003 to October 2006. Seedlings were censused monthly from March 2004 onwards 2006. Seedlings that experienced above-ground tissue mortality but resprouted were recorded. Total height (length of main stem) and stem diameters (2 cm above-ground) of seedlings were measured 18 times from April 2004 to July 2006.

During the experimental period, we measured light availability, soil moisture, soil temperature and biomass of herb and litter layers. Light availability (proportion of light reaching seedlings) was quantified using hemispherical photographs taken with a fish-eye lens (FC-E8; Nikon Corp., Tokyo, Japan) and a digital camera (CoolPix 995; Nikon Corp., Tokyo, Japan). Photographs were

taken at dawn in the summer 2004, in the centre of each subplot above the herb layer (c. 60–70 cm). Images were analysed with Hemiview software (Delta-T Devices Ltd, Cambridge, UK), and the proportion of global (direct and diffuse) radiation under the forest canopy relative to the open [global site factor (GSF)] was determined. Volumetric soil moisture content in the upper 15 cm of soil was estimated using time domain reflectometry (Soil Moisture Equipment Corp., Santa Barbara, California, USA). Data were normalized by dividing soil moisture content by maximum volumetric soil moisture content. Soil temperature was measured with thermocouples (Mezão, Lisbon, Portugal) in four random points per subplot at a depth of 3 cm. Measurements were taken after solar midday (maximum daily soil temperature). Soil moisture and temperature measurements were performed in all subplots from April 2004 to August or September 2006, with an average periodicity of seven and six times per year, respectively. We used linear regression models to simulate daily maximum soil temperature from measured soil temperature and daily maximum air temperature. Herb biomass was sampled from two random areas of 10 × 50 cm in all intact subplots and oven-dried at 70 °C to constant weight. Herb biomass was estimated at peak growth in spring of the 3 years. Litter samples, composed by senesced tree leaves, were collected from a random area of 10 × 50 cm in all subplots in 2004 and oven-dried at 70 °C until constant weight was achieved.

ANALYSIS

Environmental conditions

Differences in environmental conditions (light, soil temperature, soil moisture, herbaceous and litter biomass) between habitat types were tested with univariate analyses of variance (GLM Univariate procedure). When data were sampled in several dates, dependent data were the means of the sampling points per plot. Differences between herb biomass in the 3 years and habitat types were tested using univariate analyses of variance (GLM Univariate procedure). Data were tested for normality and homoscedasticity and transformed whenever necessary.

Growth model

Given the large measurement error around diameter measurements in comparison with diameter increments, we only analysed height data. We analysed daily growth rates (height difference/number of days between measurements) to account for different length of the inter-census periods. Growth was estimated from a saturating function driven by soil moisture, reflecting the limit on how much a plant can grow even if conditions are optimal. This is a common approach used to analyse seedling growth as a function of the limiting resource (Canham *et al.* 1999; Ibáñez, Clark & Dietze 2009). Growth was also analysed as a function of light, resprouting status, biomass of herbs and age (1st, 2nd or 3rd year) of the seedling. For seedling i at time t , we have:

$$\text{Likelihood: Growth}_{i,t}^{\text{observed}} \sim \text{Normal}(\text{Growth}_{i,t}, \sigma^2)$$

$$\text{Process model: Growth}_{i,t} = g_i M_{i,t} + \alpha \text{herb}_{i,\text{year}(t)} + \text{age}_{i,t}$$

g_i is the individual's maximum growth rate, drawn from a log-normal distribution to ensure positive values and estimated as a

function of habitat (canopy or open), light and resprouting status (yes = 1, no = 0):

$$g_i \sim \text{LogNormal}(\mu_i, \sigma_g^2)$$

$$\mu_i = \beta_1 \text{habitat}(i) + \beta_2 \text{light}_i + \beta_3 \text{resprout}_i$$

$M_{i,t}$ represents the saturating function along the soil moisture gradient:

$$M_{i,t} = \frac{\text{soil } m_{i,t} - s_{0i}}{\theta_i + \text{soil } m_{i,t}}$$

s_{0i} is the compensation point or minimum level of soil moisture necessary to start growth and θ is the half-saturation constant, that is, the level of soil moisture necessary to reach half of the maximum growth rate. Given the complexity of the models, we used a Bayesian approach to estimate parameter values, from prior distributions, all with non-informative values (Clark *et al.* 2005). All variances and fixed effects coefficients were estimated as: $(1/\sigma_g^2) \sim \text{Gamma}(0.01, 0.01)$ and α_* , age_* and $\beta_* \sim \text{Normal}(0, 10\,000)$. The compensation point and half-saturation constant were estimated at the seedlings level (to account for the large variability observed in the data) as $s_{0,i} \sim \text{Normal}(ms_0, \sigma_{s_0}^2)$ and $\theta_i \sim \text{Normal}(m\theta, \sigma_\theta^2)$ with ms_0 and $m\theta \sim \text{Uniform}(0, 100)$, and $\sigma_* \sim \text{Uniform}(0, 100)$. We then used the predicted growth, $\text{Growth}_{i,t}$, at each iteration of the model simulations to estimate survival (see section below). With this approach, we avoided unidirectional links (only growth informs survival), were able to integrate all the information collected on the seedlings into one analysis and accounted for the correlation in demographic rates, that is, growth and survival rates (Clark *et al.* 2010; Ibáñez & McCarthy-Neumann, in press).

Survival model

Survival was estimated at each census interval as a function of previous growth, a proxy for the seedling's carbon balance, and as a function of environmental variables (soil moisture, soil temperature, herb biomass and litter), as these variables may also affect survival when extreme conditions took place even if growth rates were high. The fixed effects associated with growth and herb biomass were estimated for each habitat, canopy and open, as exploration of the data seemed to suggest a differential effect of the herb layer between the habitats (data not shown). Survival for seedling i at census time t is given by $y_{it} = 1$ if the seedling is alive, and 0 if it is dead. The probability that seedling i is alive at time t is estimated as a Bernoulli process with probability of survival P :

$$Y_{i,t} \sim \text{Bernoulli}(P_{i,t})$$

$$\begin{aligned} \text{logit}(P_{i,t}) = & k_1 + k_2 \text{Soil } m_{i,t} + k_3 \text{habitat}(i) \text{Growth}_{i,t} + k_4 \text{litter}_i \\ & + k_5 \text{Soil temp}_{i,t} + k_6 \text{habitat}(i) \text{herb}_{i,\text{year}(t)} \end{aligned}$$

where all parameters were estimated as $k_* \sim \text{Normal}(0, 10\,000)$.

To test for significant effects of the explanatory variables, we looked at the 95% credible interval (CI) around the parameter estimates, and to test for the differences among the effect of age, habitats or species, we looked at the parameters differences, for example $\beta_{1,\text{canopy}} - \beta_{1,\text{open}}$, and considered them statistically

significant if the 95% CI around the parameter mean or around the difference did not include zero. We performed model simulations to predict seedling survival and growth with changing soil moisture conditions and, in the case of survival, with a 3 °C increased temperature. For that, we used draws from the posterior distributions, using the parameters' means, variances and covariances, allowing us to propagate the uncertainty associated with the estimation of the parameters. For these simulations, we used the average light, herb biomass and litter estimated for each habitat and predicted growth values estimated under those conditions.

To assess the canopy effect on growth and survival, we compared predicted values obtained for the two habitats, open and canopy, under different soil moisture levels and, in the case of survival, under a high temperature (+3 °C) scenario. Comparisons were made at the average predictions obtained for the reference habitat, that is, open (Garrett & Zeger 2004). Effect of canopy (EC) was calculated as the ratio of the probabilities of reaching a survival or growth rate as high or higher than those averaged for the reference habitat (0.5 for the reference habitat). Values of EC > 1 indicate a positive effect of the canopy, whereas values < 1 indicate a decrease in survival under the canopy.

Models were run in OpenBUGS (Thomas *et al.* 2006) to estimate the parameters (75 000 iterations). Three chains were monitored for convergence, and after the burn-in period was discarded (*c.* 50 000 iterations) and autocorrelation of the estimates eliminated (by thinning every 100th iteration), we calculated posterior means, standard deviations and 95% credible intervals.

Results

Seedlings growing under the canopy and in the open were exposed to varied light, soil temperature and soil moisture conditions (Table 1). The proportion of light reaching the seedlings and soil temperature were significantly lower ($P < 0.05$) under the canopy than in the open. The highest differences in soil temperatures observed between canopy and open areas were in summer, with a minimum difference of 15.5 °C. Canopies buffered soil temperature, lowering soil temperatures in summer and slightly increasing the minimum temperatures in winter (Fig. 1a). Overall,

Table 1. Values for the environmental variables measured under the canopy of trees and in the open

Variables	Under the canopy	Open areas
Light	0.22 ± 0.01a (0.14–0.43)	0.76 ± 0.04b (0.47–0.92)
Soil temperature (°C)	20.41 ± 0.27a (3.53–38.89)	27.14 ± 0.43b (0.03–56.83)
Soil moisture (normalized) (%)	0.39 ± 0.05 (0.17–0.96)	0.40 ± 0.05 (0.16–0.93)
Herbaceous biomass (g m ⁻²)	141.32 ± 58.23 (33.30–234.41)	194.21 ± 72.69 (61.84–312.47)
Litter biomass (g m ⁻²)	175.96 ± 33.12a (13.70–525.80)	19.32 ± 6.06b (0–82.20)

Means ± SE (min. and max. range). Different letters show significant differences at $\alpha < 0.05$.

average soil moisture content did not differ significantly between the two habitat types (Table 1). However, soil moisture varied within the year and was generally higher in the open than under the canopy during winter and early spring (Fig. 1b), with no differences in the summer. Herb biomass was lower under the canopy than in the open, although not significantly (Table 1). Herb biomass varied significantly between years ($F_{2,27} = 38.91$, $P < 0.001$). In 2005, a drier year, biomass production was very low (45 ± 9 g m⁻²), five times lower than in 2004 (269 ± 32 g m⁻²) and four times lower than in 2006 (179 ± 21 g m⁻²). Litter biomass was nine times higher under the canopy than in the open (Table 1).

GROWTH MODEL

The basal maximum growth rates (β_1) were similar for the two species, but within each species, these were higher in the canopy habitat (Table 2; Fig. 2). Increasing light

Table 2. Results for the growth model of *Quercus suber* and *Q. ilex* seedlings

Variables	<i>Q. suber</i>	<i>Q. ilex</i>
σ^2	0.00038 ± 0.00001 (0.00036, 0.0004)	0.0004 ± 0.000011 (0.0004, 0.00049)
Herb biomass (α)	5.02 E ⁻⁷ ± 4.36 E ⁻⁶ (-8.08 E ⁻⁶ , 9.26 E ⁻⁶)	-5.51 E ⁻⁶ ± 4.38 E ⁻⁶ (-1.38 E ⁻⁵ , 3.41 E ⁻⁶)
Age 1st year	0.025 ± 0.0008a (0.023, 0.0269)	0.026 ± 0.001a (0.024, 0.028)
Age 2nd year	0.02 ± 0.0005a (0.019, 0.021)	0.022 ± 0.0006a (0.021, 0.023)
Age 3rd year	0.04 ± 0.001b (0.038, 0.042)	0.04 ± 0.001b (0.038, 0.043)
Maximum growth rate (g)		
Under canopy (β_1 , canopy)	-3.27 ± 0.18a (-3.58, -2.85)	-3.16 ± 0.19a (-3.58, -2.82)
Open (β_1 , open)	-5.3 ± 0.545b (-6.24, -4.42)	-4.26 ± 0.52b (-5.39, -3.34)
Light (β_2)	4.36 ± 0.59 (3.24, 5.62)	2.38 ± 0.65 (1.19, 3.76)
Resprout status (β_3)	-0.33 ± 0.12 (-0.57, -0.08)	-0.49 ± 0.23 (-0.95, -0.04)
σ_g^2	0.94 ± 0.0.09 (0.77, 1.14)	0.84 ± 0.12 (0.66, 1.1)
Compensation point		
ms_0	3.23 ± 0.23a (2.75, 3.68)	3.3 ± 0.33 a (2.69, 4.03)
σ_{s0}^2	0.9 ± 0.28 (0.56, 1.47)	0.05 ± 0.37 (0.01, 0.79)
Half-saturation constant		
$m\theta$	61.13 ± 0.01a (61.09, 61.15)	50.07 ± 0.33b (49.43, 50.65)
σ_0^2	0.006 ± 0.001 (0.004, 0.009)	0.058 ± 0.65 (0.01, 1.36)

Posterior means ± SD (95% credible intervals). Values in bold mean statistically significant effect of the variable (95% CI did not include zero). Different letters denote statistically significant differences between ages or between habitats (β_1) within each species, or differences between species (ms_0 , $m\theta$).

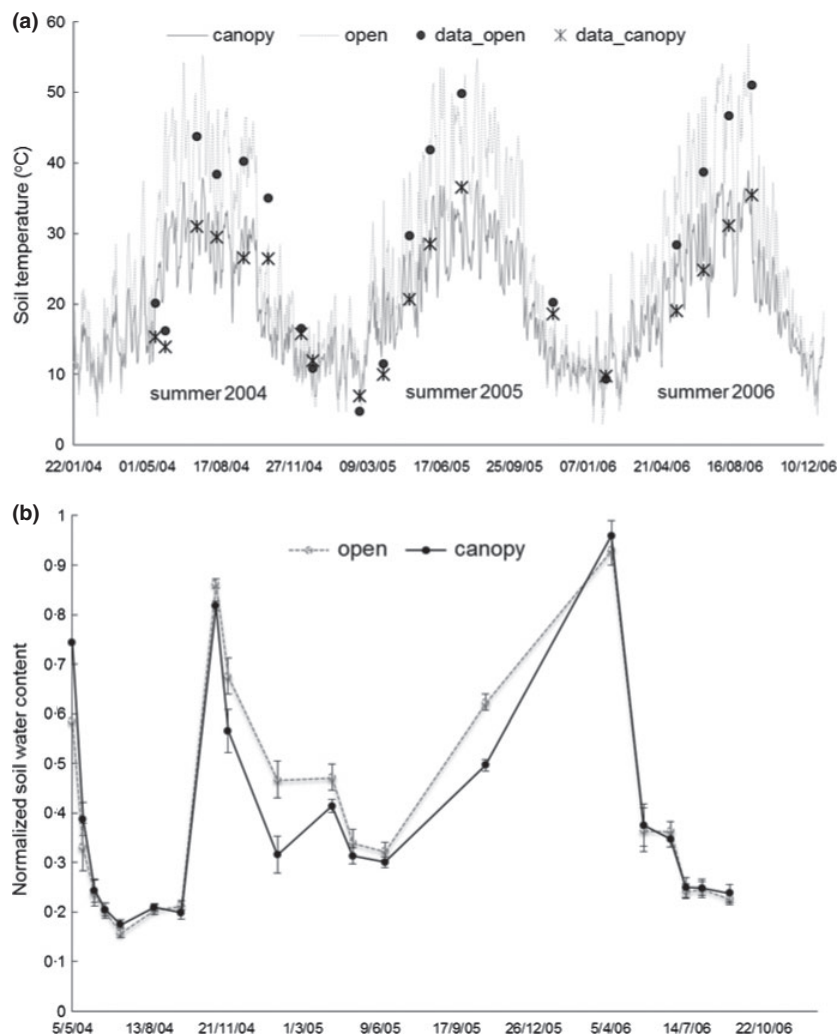


Fig. 1. (a) Modelled daily maximum soil temperature ($^{\circ}\text{C}$) under the canopy and in the open during the experiment. Measured soil temperature under the canopy (data_canopy) and in the open (data_open) is presented for comparison; (b) Normalized soil water content obtained by dividing by the maximum volumetric soil water content (mean \pm SE) under the canopy and in the open from May 2004 to September 2006.

intensity had a significant positive effect, determining the maximum growth of both species and maximum growth rates decreased significantly with resprouting for *Q. suber* (Table 2; Fig. 2). Herb biomass had no effect on the growth of *Quercus* seedlings (Table 2; Fig. 2). The two species had very similar low compensation points (ms_0), but the half-saturation constant ($m\theta$) was higher for *Q. suber* (Table 2). Growth of seedlings of both species increased significantly with age from year 2 to 3, but no differences were observed between the two *Quercus* species (Table 2).

SURVIVAL MODEL

Survival of both *Quercus* species was positively associated with increasing levels of soil moisture and negatively associated with increasing soil temperature (Table 3; Fig. 2). Herb biomass affected *Q. suber* survival negatively under the canopy and in the open habitats, but *Q. ilex* survival was only negatively affected by herbs in the open (Table 3; Fig. 2). Increasing litter biomass did not affect survival of both *Quercus* species (Table 3; Fig. 2). Previ-

ous growth had a positive effect in the survival of *Q. ilex* and negative effect for *Q. suber* (Table 3; Fig. 2).

PREDICTED GROWTH AND SURVIVAL

The predicted growth rates were considerably higher for both species and habitat types at high soil moisture (35%) than at average (15%) or low (5%) soil moisture levels (Fig. 3). Growth rates increased with age, in particular from the second to the third year (Table 3; Fig. 3). There was a large overlap in growth rates between the two habitat types, but in general, predicted growth rates were always higher in the open environment (Fig. 3). Also, although predicted growth was not significantly different between species, *Q. suber* had higher growth rates than *Q. ilex* at high soil moisture levels (Fig. 3).

At average and lower soil moisture levels, the survival of both *Quercus* species decreased significantly. The negative effect of herbs on seedling survival of *Q. suber* was significant in the two habitats, and it took effect at average or lower soil moisture, becoming less important as soil moisture increased (Fig. 4). Herb biomass decreased

Table 3. Results of the survival model for *Quercus* seedlings

Variable	<i>Quercus suber</i>	<i>Quercus ilex</i>
Intercept (κ_1)	4.11 ± 0.18 (3.75, 4.45)	3.93 ± 0.19 (3.52, 4.3)
Soil moisture (κ_2)	0.09 ± 0.012 (0.068, 0.119)	0.07 ± 0.01 (0.05, 0.104)
Previous growth (κ_3)	-0.95 ± 0.21 (-1.35, -0.57)	2.73 ± 0.16 (2.45, 3.03)
Litter (κ_4)	-0.008 ± 0.004 (-0.01, 0.001)	0.003 ± 0.006 (-0.009, 0.017)
Soil temperature (κ_5)	-0.03 ± 0.003 (-0.04, -0.028)	-0.04 ± 0.004 (-0.05, -0.03)
Herb biomass (κ_6)		
Canopy ($\kappa_{6, \text{canopy}}$)	-0.002 ± 0.0007 (-0.0039, -0.0009)	-0.0017 ± 0.001 (-0.003, 0.0003)
Open ($\kappa_{6, \text{open}}$)	-0.001 ± 0.0004 (-0.0025, -0.0006)	-0.0017 ± 0.0005 (-0.002, -0.0007)

Posterior means ± SD (95% credible intervals). Values in bold mean statistically significant effect of the variable (95% CI did not include zero).

significantly the probability of survival of *Q. ilex* in the open, at average or lower soil moisture levels, but not under the canopy habitat (Fig. 4). During the 3 years, soil moisture was below the average values of 15% during c. 5, 7 and 7 months in 2004, 2005 and 2006, respectively.

We also assessed the role of the canopy effect (EC; Fig. 5) on seedling growth and survival at different soil moisture levels and, in the case of survival, under a scenario of increased temperatures (+3 °C). Canopies had an overall detrimental effect on growth rates, an effect that increased with increasing soil moisture. When growing under a tree canopy, the probability of reaching growth rates similar to those averaged in the open decreased by 23% for *Q. suber* and by 33% for *Q. ilex* at low soil moisture conditions and by 76% and 77%, respectively, under high soil moisture. With respect to survival, the canopy nurse effect was particularly relevant under higher temperatures and low soil moisture. Most EC values at current temperatures were around 1 (no effect; Table 4), but under a +3 °C climate scenario and low soil moisture (5%), nurse canopies increased the probability of survival in comparison with average open rates by 12% for *Q. suber* and 20% for *Q. ilex*.

Discussion

Most studies of facilitation suggest positive canopy nurse effects on tree recruitment due to the amelioration of stressful conditions, for example reduced temperature (Puerta-Piñero, Gómez & Valladares 2007), or increase in resources, for example water through hydraulic lift (Brooker *et al.* 2008). Our sowing experiment and integrated model showed that the facilitative role of tree canopies in the recruitment of *Q. suber* and *Q. ilex* seedlings was more complex. In the low range of soil moisture

Table 4. Effect of Canopy (EC) on seedling daily growth rates (at 3 years old) and survival probabilities (to 3 years of age), under different scenarios of temperature (survival) and soil moisture (growth and survival)

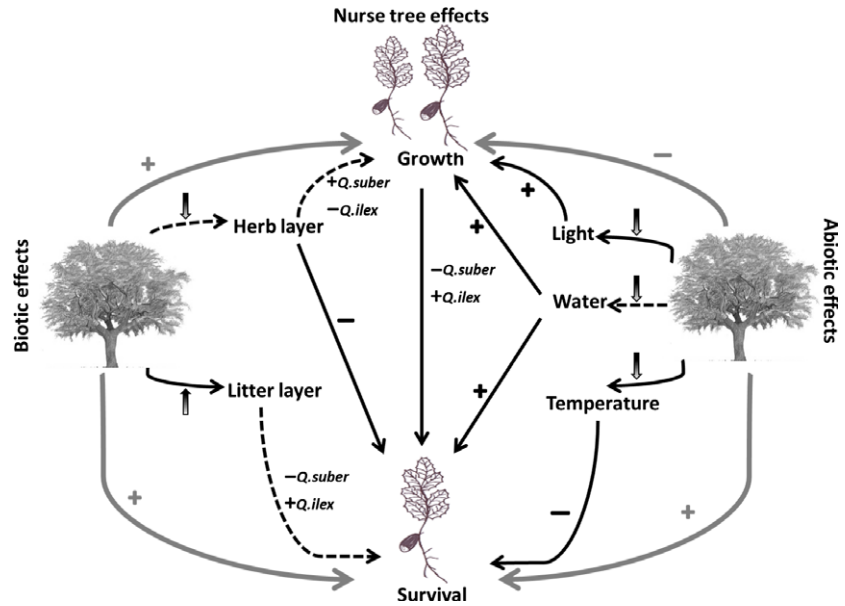
Scenario	<i>Quercus suber</i>	<i>Quercus ilex</i>
Growth (age 3)		
Low soil moisture (5%)	0.77	0.67
Average soil moisture (15%)	0.33	0.34
High soil moisture (35%)	0.24	0.23
Survival – current temperatures (5.6–43.3 °C)		
Low soil moisture (5%)	0.99	1.09
Average soil moisture (15%)	1.05	1.01
High soil moisture (35%)	1.03	0.98
Survival – high temperatures (current +3 °C)		
Low soil moisture (5%)	1.12	1.20
Average soil moisture (15%)	1.13	1
High soil moisture (35%)	1.06	0.94

Values >1 indicate a facilitating effect of canopies over the open habitat, values <1 indicate a detrimental effect of canopy habitat on growth or survival when compared with seedling performance in the open.

levels (below 15%), a condition that occurred for c. 6 months of the year, nurse canopies had a positive effect on seedling survival (Fig. 2). In addition to ameliorating the effects of high summer temperatures, nurse canopies also reduced herb biomass and indirectly facilitated seedling survival by reducing competition. The canopy nurse effect was predicted to be even stronger in the higher temperature scenario (+3 °C). These results are particularly important for the Mediterranean ecosystems, where soil moisture is generally low from late spring to beginning of autumn and where high temperatures and a lengthening of the drought season have been forecasted under climate change (Costa, Santos & Pinto 2012).

The tree canopy facilitated *Quercus* species survival directly through decreasing high temperatures, particularly when soil moisture began to decrease below average values (Fig. 4). Maximum soil temperature differences between the canopy and open habitats were high, c. 15.5 °C, during the three summers of the experiment. High temperatures can directly inflict physiological damages to seedlings (e.g. proteins begin to denature), but also increase the need of seedlings to dissipate heat energy through transpiration (Kolb & Robberecht 2006). Survival of *Q. suber* and *Q. ilex* seedlings was found to be higher in the shade than in areas with high light levels, even without water limitation, this was most probably due to decreased water needs (i.e. transpiration) and alleviation of heat stress (Gómez-Aparicio *et al.* 2008). We found that canopy cover decreased soil moisture during winter and early spring but had no effect on soil moisture during late spring and summer when environmental conditions are critical for seedling survival, a pattern also found in other studies (e.g. Valladares & Pearcy 2002; Quilchano *et al.* 2008; Cuesta *et al.* 2010). Even if soil moisture levels were similar between canopy and open

Fig. 2. Graphical representation of the nurse canopy tree effects on seedlings indicating biotic and abiotic interactions. Upward thick arrows indicate an increase in the factor under the the canopy compared with open areas, downwards thick arrows indicate a decrease. Solid thin arrows denote significant effects; dashed thin arrows indicate non-significant results. Grey arrows represent the overall effect of nurse canopy trees on growth and survival of seedlings. Positive signs indicate a beneficial effect (through facilitation, available resources or suitable conditions), and negative signs indicate detrimental effects (through competition, lack of resources or heat stress). If species names are not indicated that is because both species showed the same pattern.



habitats during spring and summer, seedling water demand would have been higher in the open habitats, and the water availability is not likely to reach the levels required by seedlings under more extreme conditions.

The indirect facilitative effects of tree canopy on seedlings resulted from their interaction with herbs. Herbs are highly competitive for water in the Mediterranean ecosystems (Rey Benayas *et al.* 2002, 2005; Cuesta *et al.* 2010). Here, we show that herbs were linked to decreased recruitment of both *Quercus* seedlings, especially under low moisture and in the open habitat. Interestingly, even if seedlings benefited directly from high soil water availability in wet years, the increased competition from the herb layer in such years still had an overall net negative effect on seedling survival.

Previous growth was positively associated with the survival of *Q. ilex* and negatively with the survival of *Q. suber* seedlings. Previous growth has been used as a surrogate of whole plant carbon balance (Kobe *et al.* 1995; Kobe & Coates 1997), reflecting the trade-off between the capacity to grow quickly and the ability to tolerate growth suppression (Kobe & Coates 1997; Zavala *et al.* 2011). Both *Quercus* species are slow-growing and stress-tolerant species, *Q. suber* had a higher growth capacity when not limited by water, but was also less tolerant to growth suppression than *Q. ilex*.

Our model also disentangled the effects of temperature and light on seedling growth and survival of the two species. Increasing light has been pointed out as responsible for decreasing seedling survival, mainly due to its covariation with increasing stress conditions (e.g. Gómez-Aparicio, Valladares & Zamora 2006; Puerta-Piñero, Gómez & Valladares 2007). In our study, as we had direct temperature data, light was only included in the growth submodel. As expected, light had a beneficial effect on growth, better reflecting the actual effect of light on recruitment and not

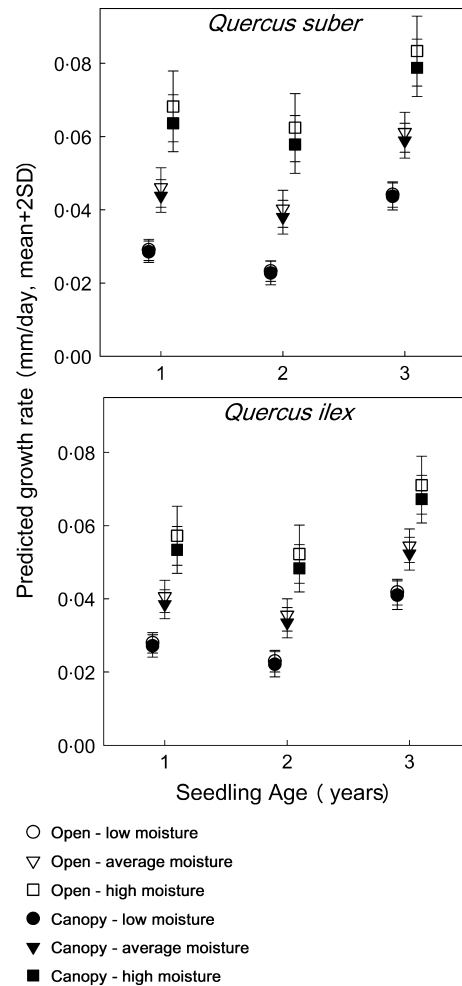


Fig. 3. Predicted growth rates for *Quercus suber* and *Q. ilex* from one to 3 years old at low (5%), average (15%) and high (35%) soil moisture for the two habitats, open and canopy. Intervals that do not overlap are significantly different.

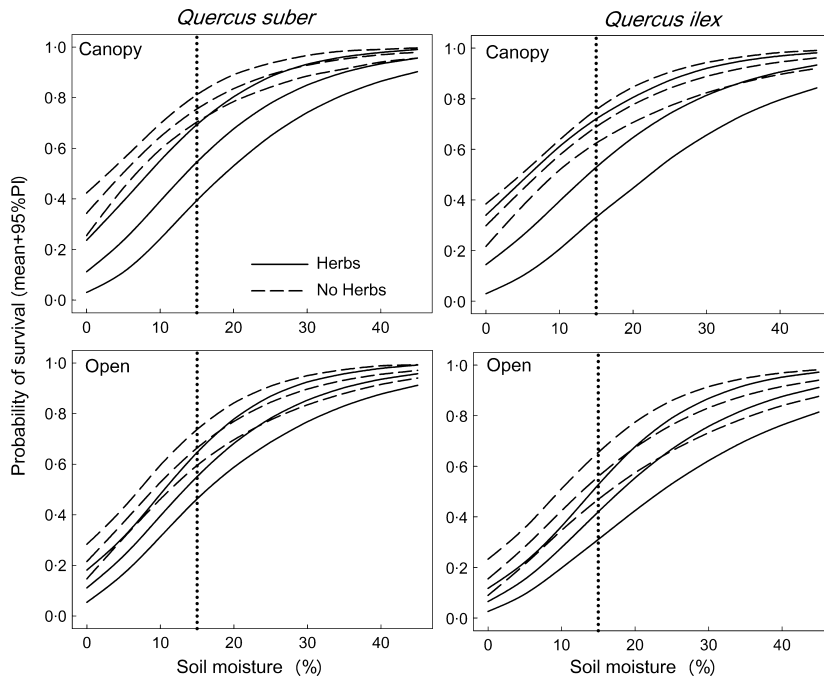


Fig. 4. Probability of survival up to 3 years (mean ± SD) for *Quercus suber* and *Q. ilex* under the canopy and in the open, with and without the presence of herbs. Vertical lines represent average soil moisture (c. 15%) in the plots.

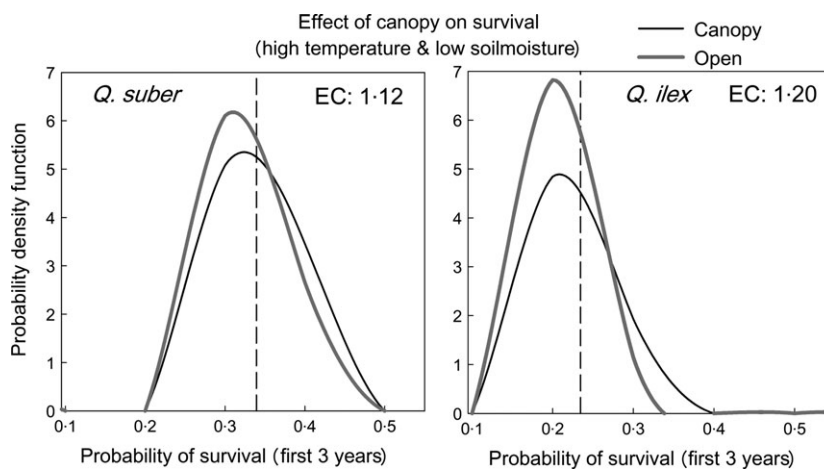


Fig. 5. Predicted survival probability density functions (pdf) showing the effects of canopy (EC) when compared at the average survival rate in the open habitat (vertical lines). EC is calculated as the ratio of the areas to the right of the vertical line.

its confounding effect with temperature. Simulation of overall growth, however, showed higher growth rates in the open where light levels are higher, especially at high soil moisture levels (Table 4; Fig. 3). Maximum growth rates of the seedlings, that is, growth under optimal conditions, were favoured under the tree canopy; probably due to decreased water stress caused by lower temperatures and reduced competition with herbs.

The first years of seedling establishment are the most critical stage of tree recruitment (Castro *et al.* 2004; Zavala *et al.* 2011). *Quercus* species in early stages invest much more carbon in below-ground parts (Verdaguer *et al.* 2001), a pattern that changes once seedlings are established. During the establishment phase, even the positive effects of a first wet year on survival and growth can be cancelled out by subsequent dry years (Gómez-Aparicio *et al.* 2008). The significant growth increase in

both *Quercus* species seedlings from the second to the third year may be related to the growth-limiting effects of the dry second year and may also indicate a higher allocation of resources to stem growth on the third year, indicating that the establishment of seedlings was occurring at this time. While soil moisture and temperature are critical factors determining survival during the establishment phase, light, which influences growth rate (along with soil moisture), may become more important in later plant life stages (Niinemets 2006; Zavala *et al.* 2011).

MANAGEMENT IMPLICATIONS

Our results show that tree canopy facilitated survival of *Quercus* seedlings, especially at low soil water levels. Adding to the evidence of the facilitative role of shrubs (e.g. Gómez-Aparicio *et al.* 2004), our results suggest that

tree canopy facilitation should also be considered in the management plans of the Mediterranean *Quercus* woodlands. These woodlands are threatened by a decrease in the density of mature trees (e.g. Bugalho *et al.* 2011), requiring an additional effort to increase regeneration. Facilitation of seedling recruitment by tree canopies in forest management practices may be essential to ensure proper regeneration of these woodlands, especially under forecasted climate change scenarios of increasing high temperatures and drought. Our results also show the negative effect of herb biomass on seedling survival. Active management of herbs (e.g. mowing, controlled grazing), particularly in years with wet springs, may increase *Quercus* seedling survival as has been shown for several Mediterranean species (Rey Benayas *et al.* 2005; Cuesta *et al.* 2010).

Management plans promoting maintenance of a healthy adult tree canopy cover may be critical to assure regeneration of the Mediterranean evergreen oak woodlands. Also, using pioneer species, such as pines, may promote *Quercus* seedling establishment (Vallauri, Aronson & Barbero 2002; Gómez-Aparicio *et al.* 2009) in areas without mature deciduous tree canopies. For example, in Portuguese *Q. suber* woodlands, *Pinus pinea* L. has been used as a nurse species. In addition to its ecological role, *P. pinea* also provides a regular source of income to landowners (e.g. pine nuts), beyond that generated by cork (Coelho & Campos 2009). Lastly, a better understanding of the feedbacks and interactions between biotic responses and environmental change are needed to further improve our predictive and management capabilities to enhance regeneration of the Mediterranean woodlands under climatic change.

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