Facilitation of holm oak recruitment through two contrasted shrubs species in Mediterranean grazed woodlands.

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Running head: Tree recruitment in encroached oak woodlands

Abstract

Question: Regeneration failure and gradual tree dieback are major threats for the persistence of savanna-like grazed oak woodlands. Current research has argued that the scarcity of "safe sites", in particular shrubs, is the main cause of the lack of effective tree recruitment. But can different shrub species be considered as safe sites generally? Do two distinct shrub species, with contrasted life strategies, affect several life stages of tree regeneration in similar ways or do they specifically influence the recruitment process?

Location: Holm oak woodlands of SW Iberian Peninsula (40° 02'N; 06°06'W)

Methods: We surveyed densities of recently emerged and survived seedlings as well as small and large saplings during two consecutive years in 40 sites that were independently managed comparing plots encroached by either *Cistus ladanifer* (a shallow-rooted shrub with reported allelopathic compounds that forms thick populations) or *Retama sphaerocarpa* (a N_2-fixing deep-rooted shrub that forms scattered populations) versus their respective control plots (without shrubs). To assess the effect of mature trees and both shrub species on the performance and survival of recently emerged oak seedlings, we established an acorn sowing experiment in the same surveyed microhabitats (open spaces, shrub, tree and tree-shrub).

Results: The survey showed that both shrubs species had a positive effect at early recruitment stages. At later life stages, this effect weakened under *Cistus* whereas it strengthened under *Retama*. The acorn sowing experiment showed that both shrub species buffered abiotic conditions and enhanced seedling functioning similarly, but *Retama* enhanced seedling survival to a higher extent than *Cistus*.

Conclusions: The two shrub species impose a specific template that is able to affect the long-term dynamics of Mediterranean oak woodlands. *Cistus* shrubs are effective to protect seedlings physically against herbivores and facilitate early survival, but may compete with older stages of oak regeneration. By contrast, *Retama* shrubs exert a stronger biological facilitation and guarantee log-term persistence of surviving seedlings. We argue that improved understanding of the effectiveness of different nurse plants and their contrasting factors is of major interest for the conservation and restoration of degraded oak woodlands.

Keywords: Dehesa; *Cistus ladanifer*; *Retama sphaerocarpa*; *Quercus ilex*; nurse plant; shrub encroachment.

Introduction

Many oak woodlands are managed for livestock husbandry worldwide (Grove & Rackham 2003; McCreary 2004; Salehi et al. 2008; Bergmeier et al. 2010). In many cases, an open forest cover is created through tree thinning and shrub clearing to enhance pasture productivity. The result is a savanna-like landscape that consists of a pasture matrix with isolated oak trees and a sparse shrub cover. The most outstanding example of such landscapes in Europe is the dehesa in the Southwest of the Iberian Peninsula, which covers nearly 3.5 million ha (Moreno & Pulido 2009). The high structural diversity generated in these landscapes has been associated with a high biological diversity (Díaz et al. 1997). Holm oak constitutes the most important tree species of this system in ecological and economical terms (Blanco et al. 1997). It is considered a keystone species of Mediterranean Basin forests (Terradas 1999; Blondel et al. 2010) because of its importance for the maintenance of ecosystem services (Plieninger & Wilbrand 2001; Marañón et al. 2009; Gea-Izquierdo et al. 2010; Howlett et al. 2011; Plieninger et al. 2011). However, these woodlands, as in other Mediterranean climates zones (e.g. California, Tyler et al. 2006) and agricultural landscapes worldwide (Gibbons et al. 2008), are under threat by a lack of effective regeneration, accompanied by a gradual dieback of adult trees. Studies of demographic structures of tree populations, based on age or size classes, have confirmed an almost complete lack of juvenile classes (Pulido et al. 2001; Plieninger et al. 2003; Ramírez & Díaz 2008). This insufficient natural oak regeneration has been explained mainly by deficient seed dispersal to suitable sites for seedling establishment and survival (Pulido & Díaz 2005). Thus, it is of particular interest for current research on the dynamics of oak savannas to identify effective "safe sites" for tree regeneration to guarantee the system stability and likelihood of persistence.

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78   Temporal abandonment, and subsequent shrub encroachment, has been proposed as a

79   cost effective tool, compared to artificial plantation, to enhance natural recruitment of

80   trees (Pulido et al. 2010; Ramírez & Díaz 2008). Shrubs may play a major role for tree

81   regeneration both at the dispersal and post-dispersal stages (Muñoz & Bonal 2007;

82   Puerta-Piñero et al. 2007; Puerta-Piñero et al. 2010; Gómez et al. 2008). Dispersers, for

83   example rodents (Muñoz & Bonal 2007; Smit & Verwijmeren 2011) and jays (Gómez

84   2003), transport acorns preferentially towards shrubs partly because they can forage and

85   feed more safely from predators (Muñoz & Bonal 2007). Many of the seeds that they

86   transport under shrubs are consumed, but – compared to other microhabitats – the

87   surviving acorns have a higher probability to exceed a safe threshold for early

88   regeneration (Gómez et al. 2008; Perea et al. 2011; Puerta-Piñero et al. 2010). The

89   prevention of desiccation and the improvement of germination and emergence

90   conditions of cached acorns have been shown to be the most prominent facilitative

91   effects of shrubs at this stage (Vander Wall 2001; Gómez-Aparicio et al. 2005; Puerta-

92   Piñero et al. 2007).

93

94   Once a seedling has emerged, shrubs can facilitate seedling survival mainly through

95   protection against herbivory and improved suitable abiotic conditions. This is of great

96   importance as most mortality of tree seedlings occurs after the first summer season

97   (Gómez 2004). Hence, shading from direct radiation can reduce water stress and prevent

98   the desiccation of early recruits (Gómez-Aparicio et al. 2004; Smit et al. 2008)

99   influencing positively seedling survival and growth (Puerta-Piñero et al. 2007).

100   However, it has been argued that the outcome may differ depending on the shrub

101   species that is present (Gómez-Aparicio et al. 2004; Puerta-Piñero et al. 2007). Shrub

102   species with contrasted key functional traits may affect the availability of abiotic

103   resources or the impact on biotic stresses differently, resulting in a potentially different

104   outcome (Eldridge et al. 2011). For instance, unpalatable shrubs, or high density shrub

105   formations, may be more effective against herbivore pressure and thus enhance tree

106   survival (Smit et al. 2006). However, relatively few studies have explicitly examined

107   this effect in natural communities rather than in transplanting experiments.

108

109   In addition, it is uncertain if the differential effect among shrub species affects similarly

110   all life stages of the facilitated plant or if it varies during its ontogeny. Shrub species

111   whose above and below-ground traits contributed to competition, while only above-

112   ground traits contributed to facilitation can be beneficial at early regeneration stages but

113   detrimental at older ones (Sthultz et al. 2007). In this context, surprisingly, few studies

114   have assessed deviations from this evidence such as the effect of a shrub species whose

115   below-ground traits contributed mainly to facilitation (Kulmatiski et al. 2008; Gómez-

116   Aparicio et al. 2005; Puerta-Piñero et al. 2006).

117

118   In this study, we aim to determine if two different shrub species (*Cistus ladanifer* and

119   *Retama sphaerocarpa*, with contrasted life strategies: a competitor and a facilitator of

120   neighboring vegetation, respectively; Rivest et al. 2011; Rolo & Moreno 2011) enhance

121   oak recruitment in Iberian dehesas in comparable ways and guarantee thus the long-term

122   persistence of the land-use agro-system. We assessed shrub effects from early

123   recruitment stages to the large sapling stage. This approach would comprise most of the

124   valuable information because of the small differences on survival after the sapling stage.

125   Studying different recruitment stages, rather than inferring recruitment failure through

126   indirect studies of tree population age structure, would allow us to explain the processes

127   that have led to the observed age structure and assess effectively the effect of different

128   shrub species on the regeneration process.

129

130   We address three specific questions 1) Is there a consistent beneficial effect of shrub

131   cover on the different recruitment stages (seedlings and small and large saplings)?; 2)

132   Do the two shrub species offer similar conditions (physical protection and/or favorable

133   abiotic conditions) and impact the process of seedling survival similarly?; 3) Are these

134   effects dependent on species-specific, shrub-specific and life-stage specific

135   combinations? We hypothesize that *Cistus,* a competitor for soil resources, may impose

136   a detrimental effect on all stages of tree regeneration. *Retama*, a N_2-fixing shrub with

137   reported beneficial effect on soil properties, would facilitate both early and late stages of

138   tree regeneration. However, the contrasted physiognomy of each shrub population (thick

139   vs sparse, respectively) could additionally determine crucial factors, such as browser or

140   disperser activity, and modulate strongly this outcome. Finally, we expect that the effect

141   of shrubs would be more conspicuous beyond the tree canopy where tree regeneration is

142   more vulnerable to the lack of "safe sites".

143

144   **Material and Methods**

145   *Study area, system and species*

146   The study was conducted in the north of the Extremadura region of central western

147   Spain (39º 27'– 40º12' N; 05º 90'– 06º 30' W), in flat or gently sloping (3–5%) terrain,

148   located at an altitude of 400 –500 m a.s.l. The soils are oligotrophic and acidic and are

149   classified as Chromic Luvisols and Dystric Cambisols (IUSS Working Group WRB

150   2006). The climate of the area is Mediterranean with hot, dry summers and mild, rainy

151 winters. Mean annual precipitation and temperature are 546.8 mm and 15.5 ºC (data

152 taken from the closest weather stations, 10 km away from the furthest site).

153 The dominant vegetation in the region is a savanna-like open woodland, which is

154 dominated by a scattered holm oak overstory (*Quercus ilex*) with native pastures as

155 understory, and occasionally a sparse to dense shrub layer, mostly represented by

156 *Retama sphaerocarpa* (L.) Boiss and *Cistus ladanifer* (L.) (hereafter *Retama* and *Cistus*,

157 respectively). Pasture is the main fodder source for livestock in this system, acorns and

158 tree foliage are also important fodder sources, but shrubs are almost not consumed apart

159 of fruits (Olea & San Miguel 2006). *Cistus* is a drought-tolerant semideciduous shrub,

160 with a shallow-dense root system, and often forming extensive, clumped pure stands

161 (Silva & Rego 2004). Its leaves and stems can secret a large amount of exudates with

162 allelopathic agents that inhibit the growth of competing herbs (Sosa et al. 2010). It has

163 been shown to compete with the neighboring tree overstory and herbaceous understory

164 (Rivest et al. 2011). Its suitability as a nurse plant has been widely questioned (Acacio

165 et al. 2007). *Retama* is a N fixing leafless shrub often forming monospecific scattered

166 patches and developing a dimorphic rooting system, with both lateral roots and a main

167 deep taproot (Prieto et al. 2010). This species has been shown to enhance diversity and

168 productivity pasture understory (Pugnaire et al. 1996; Rodríguez-Echeverría & Pérez-

169 Fernández 2003; Rivest et al. 2011). Pasture is dominated by annual Mediterranean

170 herbaceous species, specifically the grasses *Agrostis curtisii*, *Agrostis castellana* and

171 *Gaudinia fragilis*, the legumes *Ornithopus compressus*, *Trifolium resupinatum* and

172 *Trifolium angustifolium*, and the forbs *Bellis perennis, Helianthemum guttatum* and

173 *Raphanus raphanistrum*.

174

175 *Large scale survey of seedling and saplings*

176 We selected 40 private dehesa estates (hereafter referred as sites) across a 2500 km²

177 area to assess the patterns of oak recruitment (see Appendix S1 for relevant

178 characteristics). The average of the minimum distance between sites was 3.4 ± 0.6 (SE)

179 km. All sites were characterized by the presence of a *Q. ilex* overstory with an average

180 tree density of 26 ± 1.6 (SE) tree ha^{-1} and an average tree diameter at breast height of

181 49.5 ± 1.9 (SE) cm. Each site included two adjacent plots (ca. 1 ha) close to one another

182 (i.e. less than 100 m apart), similar in tree cover, soil type and slope, but differing in the

183 presence of shrub: one plot without shrubs (control plot) and one with the presence of

184 shrubs (encroached plot). In half of the sites (n = 20) the encroached plot was composed

185 of *Retama* (hereafter *Retama* sites), in the other half (n = 20) it was composed of *Cistus*

186 (hereafter *Cistus* sites). Encroached plots of *Retama* sites exhibited an average shrubby

187 cover of 34.0 ± 2.9 % and an average shrub height of 1.8 ± 0.1 m. Encroached plots of

188 *Cistus* sites exhibited an average shrubby cover of 77.5 ± 2.8 % and an average shrub

189 height of 1.9 ± 0.1 m. The presence of a shrub layer in the encroached plots had been in

190 all cases caused by a recent (last 10–15 years) reduction in grazing pressure, according

191 to landowners. Additionally, in each site we felled three shrubs randomly selected in the

192 encroached plot. Annual rings were measured in a stem disk sawed at the stem base of

193 each felled shrub. This value was considered as a surrogate of time of abandonment and

194 it was similar between types of sites (12.4 ± 0.7 and 11.0 ± 0.7 years in *Cistus* and

195 *Retama* sites, respectively). Grazing intensity in control plots was apparently similar

196 among sites (personal observation based on pasture height at mid summer, and the

197 generalized presence of cattle), although sites were managed by different owners and

198 surely subtle differences exist.

199 We surveyed the presence of recently emerged seedlings (with acorn attached), small

200 saplings ($\leq$ 30 cm height, with evidence of at least one bud scar) and large saplings (>

201 30 cm height, with evidence of more than one bud scar) in the 80 selected plots (40 sites

202 x 2 plots (control and encroached). In each plot we performed a 2 m wide transect,

203 starting at the base of a tree randomly selected and following a random direction. Each

204 transect was ~ 300 m long and crossed the canopies of ~ 10 oaks. Thus, four different

205 habitats were surveyed: two from control plots, beneath the tree canopy (hereafter tree)

206 and beyond the tree canopy (hereafter open spaces); and two from encroached plots,

207 beneath trees (hereafter tree-shrub) and beyond the tree canopy (hereafter shrub).The

208 surveys were performed in late spring of 2007 and 2008. We repeated the same

209 transects in autumn of 2007 and 2008 to record the survival of recently emerged

210 seedlings. At this growth stage most mortality occurs after the first summer season;

211 small and large saplings are less likely to be affected by losses through desiccation

212 (Smit et al. 2008). We performed different transects each year to minimize

213 interdependence effects, for example by recording the same sapling both years. We

214 assessed the area (length) of transects using digital orthoimages, tree cover by

215 measuring tree canopy diameters, and shrub coverage by estimating visually their

216 proportion on transects. These values were assessed to include in the statistical model

217 differences in terms of tree canopy and shrub cover in surveyed area.

218

219 *Acorn sowing experiment*

220 To experimentally assess the effects of shrubs on the process of early recruitment

221 (seedlings < 1 year old), we performed a controlled acorn sowing experiment. In

222 November of 2008 we collected ca. 5000 acorns from trees that were selected randomly

223   in two sites (two private dehesa estates, one encroached with *Cistus*, 39º57'52.3''N -

224   6º6'16.3''W, and one encroached with *Retama,* 39º56'40.5''N - 6º11'39.7''W).

225   Unsound acorns as well as acorns with weevil damage marks were discarded. All acorns

226   were stored at 4º C until further handling. In February 2009 we sowed 300 acorns in

227   each of the four microhabitats (open space, tree, shrub, and tree-shrubs) in the same two

228   sites where the acorns were collected (total: 2400 acorns, mean fresh weight 2.6 ± 0.1 g

229   (SE), ranging from 1.5 to 7.0 g). Acorns were sown in groups of 50 and placed inside

230   metal cages to avoid damage through large herbivores and mice and to exclude

231   additional seed rain. Cages were randomly distributed within each microhabitat. We

232   checked seedling emergence at the end of spring and monitored seedling survival

233   monthly until autumn rainfall started in late September.

234   A set of biophysical parameters were recorded to assess the differential effects of two

235   shrubs species on key resources needed by seedlings (microclimate and soil resources).

236   For each cage we recorded the irradiance by means of hemispherical photographs, dry

237   season air temperature and relative humidity by means of a data-logger (PCE-HT71,

238   Spain), and soil moisture in the first 40 cm by means of TDR probes (Tektronix 1502C,

239   UK) following the procedure described by Cubera and Moreno (2007). Photographs

240   were taken above each cage, approximately at 0.25 m above the ground, using a

241   horizontally leveled digital camera (Nikon Coolpix 995) and aimed at the zenith using a

242   fish-eye lens of 180º field of view (Trichon et al. 1998; Valladares & Guzmán 2006).

243   According to these authors, photographs were taken under cloudless homogeneous sky

244   conditions before sunrise. The following morphological and functional variables were

245   measured to asses direct or resources-mediated effects of shrubs on seedling

246   performance: i) the photochemical efficiency (F_v/F_m) and ii) height (cm) of each

247   seedling in the middle of the dry season; iii) the specific leaf area (SLA, cm^2 of leaf per

248 g of leaf), iv) leaf area ratio (LAR, cm^2 of leaf per g of plant), and v) stem density (g /

249 cm^3) in 10 surviving seedlings per microhabitat and site collected at the end of the

250 summer. F_v/F_m was measured by means of a Multi-Mode Chlorophyll Fluorometer

251 (OS5p, ADC Bioscientific, UK). Leaves were removed and scanned (Epson Expression

252 10000XL). Images were analyzed with Image-Pro Plus (Version 6.0) to determine leaf

253 area. All plant material was oven-dried for 72h at 60ºC and weighed.

254

255 *Data analysis*

256 Recently emerged and survived seedling and small and large sapling data (expressed as

257 the number of individuals) were analyzed using Generalized Linear Mixed Models

258 (Bolker et al. 2009). Generalized Linear Mixed Models (GLMMs) were constructed

259 separately for regeneration stages. We assume that the dependent variable (count data)

260 was Poisson distributed (log-link). Microhabitat (open spaces, tree, shrub and tree-

261 shrub) and type of shrub present (*Cistus* and *Retama*) were used as explanatory

262 variables. To account for differences in surveyed area per microhabitat and site its value

263 was included in the model as an offset (Zuur et al. 2009). The effect of sites and years

264 were considered random effects. When significant effect on the explanatory variables

265 were found, the appropriate linear contrast were set up and tested, controlling for

266 multiple comparisons. In all cases, model validity was checked by visual examination of

267 residual plots and by assessment of dispersion parameters (Bolker et al. 2009). In those

268 models in which we observed overdispersion, an observation-level random effect was

269 included to take it into account.

270

271 Subsequently, to investigate if differences between *Cistus* and *Retama* formations on

272 the number of recently emerged and survived seedling and small and large sapling

273 responded to differences on shrub cover and/on time after abandonment, four additional

274 GLMMs were constructed, based only on the surveyed data from encroached plots.

275 Model validity and assessment of dispersion parameters was done similarly to previous

276 GLMMs. The effect of sites and years were again considered random effects. To

277 account for differences in surveyed area per encroached plot we included its value in the

278 models as an offset. Additionally to shrub cover and time after abandonment, tree

279 diameter at breast height and density of previous regeneration stage were also included

280 as explanatory variables to account for tree effect and to investigate it the effect of

281 shrubs changed with aging, respectively. The density of previous regeneration stage was

282 included in each model in a step-wise manner, excluding progressively the youngest

283 regeneration stages (Smit & Verwijmeren 2011). That is, in the model regarding

284 survived seedlings we included the density of emerged seedling, for small saplings the

285 density of survived seedlings and for large saplings the density of small saplings. The

286 "lme4" (Bates and Maechler 2010) and the "multcomp" (Hothorn et al. 2010) packages

287 of the R software (R Core Development Team 2012) were used for GLMMs and

288 multiple comparison respectively.

289

290 In the acorn sowing experiment, between microhabitat differences in abiotic conditions

291 (irradiance, air temperature and humidity and soil moisture) and seedling performance

292 parameters (F_v/F_m, height, SLA, LAR and stem density) were assessed by analyses of

293 variance. Between microhabitat differences in percentages of seedling survival were

294 evaluated using Kaplan-Meier Analysis. Data were transformed, when necessary, prior

295 to analysis to comply with ANOVA assumptions. Pearson Chi-square goodness of fit

296 tests and ANOVA analysis were performed with Statistica 7.0 (StatSoft, Inc., Tulsa,

297 OK, USA).

298

Results

Large-scale survey

A considerable proportion of visited sites lacked of recently emerged seedlings in both

years of the study. On average, we did not find any recently emerged seedling on 58.1

% of the visited sites. The proportion of sites without any sapling was considerably

lower (22.2 %), but the mean number of large saplings relative to the number of mature

trees was clearly below 1 (0.41 ± 0.03), indicating a likely regeneration failure. In those

sites where we observed recently emerged seedlings, we found a significantly higher

density under shrubs, irrespective of the shrub species (Table 1), than in open spaces

(0.40 ± 0.14 and 0.14 ± 0.07 seedlings / 100 m^2 in shrub and open spaces microhabitats

respectively, P = 0.019). This positive effect was maintained during the summer

resulting in a higher density of seedlings at the end of the summer under shrubs,

irrespective of the shrub species, than in open spaces (0.19 ± 0.09 and 0.04 ± 0.02

seedlings / 100 m^2 respectively, P = 0.018). This positive effect was also maintained

during successive regeneration stages, both small and large saplings, but there were

specific differences between sites with different shrub species present (significant

interaction between type of shrub present and microhabitat, Table 1). In sites

encroached with *Cistus*, the positive effect of shrub was similar along life stages (Fig.

1). However, in sites encroached with *Retama*, the positive effect of shrubs tended to

strengthen along life stages (Fig. 1).

319

Tree presence induced a large effect on emerged and survived seedlings and small and

large sapling density (Fig. 1). Indeed, the combined positive effect of shrub and tree led

to a higher abundance of most regeneration stages under tree-shrub microhabitat than in

13

323   open spaces (Fig. 1). In sites encroached with *Cistus,* the highest densities were found

324   under tree-shrub microhabitat for all life stages (Fig.1). In sites encroached with *Retama*

325   there was also a significantly higher abundance than in open spaces for all life stages.

326   However, in these sites, compared to shrub effect (Fig.1), the effect of trees-shrub was

327   less apparent at older regeneration stages (no significant differences between tree-shrub

328   and shrub microhabitats at the small sapling stage, $P = 0.999$; and significantly lower at

329   the large sapling stage, $P = 0.031$).

330

331   Previous regeneration stage density had a marked positive effect on the number of

332   survived seedlings and small and large saplings (Table 2). Indeed, we observed a 9.9 %

333   positive difference in the number of survived seedlings per number of emerged

334   seedling, a 23.7 % positive difference in the number of small saplings per number of

335   survived seedling and a 7.2 % positive difference in the number of large saplings per

336   number of small saplings. At the large sapling stage, the effect of the previous stage

337   density was notoriously different per type of shrub species. In presence of *Retama*, there

338   was a 75.1 % positive difference in the number of large saplings per number of small

339   saplings than in presence of *Cistus*. The differences in shrub cover or time after

340   abandonment among encroached plots of *Cistus* and *Retama* sites did not show any

341   effect on most of the regeneration stages (Table 2). Exceptionally, at the small sapling

342   stage there was a marginally significant positive effect of time after abandonment,

343   yielding a 10.5 % positive difference in the number of small saplings per year increase.

344   The effect of tree size showed a positive effect on emerged seedlings but a negative

345   effect for large saplings.

346

347   *Acorn sowing experiment*

348 Microclimate characteristics

349 The presence of a woody overstory induced significant modifications on the

350 microclimatic conditions compared to that of open spaces (Table 3A). Air temperature,

351 vapor pressure deficit (VPD) and irradiance exhibited lower values under shrub, tree,

352 and tree-shrub than that of open spaces. Nevertheless, these differences were less

353 marked in sites encroached with *Retama*, showing a 10% reduction in air temperature

354 and 40 % in irradiance on average under shrub, tree, and tree-shrub than that of open

355 spaces. Soil moisture showed a significant reduction under *Cistus*, whereas under

356 *Retama* there were no differences.

357

358 Seedling emergence

359 Seedling emergence was low in all microhabitats studied and there were no significant

360 differences between microhabitats either in sites encroached with *Cistus* or *Retama*.

361 Nevertheless, it is noteworthy that maximum emergence values were found under trees

362 and tree-shrubs in both types of sites (34% and 31% respectively in sites encroached

363 with *Cistus*; 25% and 27% respectively in sites encroached with *Retama*). In contrast,

364 open spaces and shrubs showed lower values in both types of sites (22% and 21%

365 respectively in sites encroached with *Cistus*; 21% and 23% respectively in sites

366 encroached with *Retama*).

367

368 Seedling performance

369 In the middle of the dry season, seedlings growing in open spaces showed a

370 significantly lower value of F_v/F_m than those growing under shrub, tree or tree-shrub

371 microhabitats (Table 3B). Among shrub, tree or tree-shrub, there were no significant

372 differences in F_v/F_m values. Other morphological parameters also showed significant

15

373   differences between microhabitats at the end of the summer. SLA was significantly

374   lower in surviving seedlings of open spaces than under shrub, tree or tree-shrub.

375   Seedlings growing in open spaces were smaller but with denser stems than under shrub,

376   tree or tree-shrub. In addition, seedlings growing in open spaces tended to show a lower

377   LAR which, together with the higher stem density, suggests a greater investment on

378   stem tissue than leaves.

379

380   Seedling survival

381   Seedling survival at the end of summer exhibited significant differences between

382   microhabitats (Fig. 2). Open spaces showed the lowest values. Just 20 - 28% of emerged

383   seedlings in spring survived at the end of the summer in open spaces. Most of these

384   seedlings seemed to have died of desiccation, although we did not find a significant

385   reduction of soil water content in open spaces (Table 3A). Tree and tree-shrub showed

386   the same positive pattern. Under shrub there was also a positive effect on seedling

387   survival but the extent of this effect differed noticeably between *Cistus* and *Retama*. The

388   proportion of surviving seedlings was around 41 % under *Cistus*, while it was up to 90

389   % under *Retama*.

390

391   **Discussion**

392   Our results showed that both shrub species exhibited a positive influence on certain

393   patterns of oak recruitment in open Mediterranean woodlands in general. Facilitative

394   effects of shrubs on plant recruitment have been widely reported in semiarid, tropical

395   and temperate environments (Padilla & Pugnaire 2006; Brooker et al. 2008; Gómez-

396   Aparicio 2009) as well as in the dehesa system in particular (Ramírez & Díaz 2008;

397   Smit et al. 2008; Pulido et al. 2010). Experimental evidence has been confirmed at

16

398 regional scale by a recent study which established a positive relationship between shrub

399 cover or shrub species richness with regeneration densities of *Q. ilex* and other *Quercus*

400 (Plieninger et al. 2010).

401

402 The high density of seedlings under trees, both in control and encroached plots, the

403 positive effect of tree size on the number of seedling, and the low rates of seedling

404 emergence across microhabitats in the acorn sowing experiment, evidence two main

405 processes that explain in a high extent the lack of regeneration in holm oak woodlands:

406 recruitment limitation and deficient seed dispersal to safe sites (Pulido & Díaz 2005;

407 Smit et al. 2009). Although we did not separate the effect of shrubs on pre-dispersal and

408 dispersal processes explicitly, our results suggest that both shrubs had positive effects at

409 least during the dispersal stage. Seedling emergence beyond the canopy edge tended to

410 be higher under shrubs than in open spaces. This result suggests an active dispersal of

411 acorns toward shrubs, rather than a passive dispersal that is common under tree

412 canopies (Smit & Verwijmeren 2011). In the study area, holm oak mostly relies on

413 rodents for the dispersal of acorns in open woodlands (Muñoz et al 2009). In this way,

414 rodents may enhance oak reproduction success away from the mother tree by successive

415 dispersal movements to safe sites (Perea et al. 2011). But it is also likely that other

416 factors, in particular, physical protection against herbivores or, less probably,

417 prevention of acorn desiccation, were responsible for the observed differences in the

418 density of emerged seedlings between open spaces and shrubs (Gómez-Aparicio et al.

419 2004, 2005).

420

421 On the other hand, some evidence of a negative effect of shrub cover on seedling

422 occurrence and abundance has been reported in some other studies (Pausas et al. 2006;

423 Acacio et al. 2007). These authors argued that shrub presence can arrest tree recruitment

424 by worsening/impeding several regeneration processes as acorn yield, dispersal and

425 seedling survival (Acacio et al. 2007). Our results showed that the recruitment stages

426 that follow seedling survival were in most cases positively associated with shrubs.

427 However, associations were strongly species-specific, which strengthens the assumption

428 that the physical and biological environment may play a major role in this multi-phase

429 process (Gómez-Aparicio et al. 2004, 2005). This result may be of concern in many

430 other man-made ecosystems of the world that are threatened by the lack of effective tree

431 regeneration (Gibbons et al. 2008; Manning et al. 2006; Mountford & Peterken 2003;

432 Laborde et al. 2008; Steenkamp et al. 2008; Weinberg et al. 2011).

433

434 *Seedlings performed similar plastic adaptations under shrub species*

435 Seedlings showed opposite patterns on morphological parameters depending on the

436 microhabitat where they grew, suggesting adjustments to each specific abiotic

437 condition. For instance, seedlings exhibited higher values of SLA when growing under

438 tree, shrub and tree-shrub, probably to enhance light capture in the shade (irradiance 30-

439 60% of full sunlight; Table 3) (Rodríguez-Calcerrada et al. 2010). In open spaces, by

440 contrast, they seemed to invest more on stem tissue (as expressed in lower height and

441 higher wood density), probably to enhance their resistance to cavitation. During extreme

442 drought events, however, plants with high wood density may be less successful in

443 maintaining their water potential within safe margins and may be more subjected to

444 extensive embolism (Hoffmann et al. 2011). In this context, we found that seedlings

445 growing in open spaces, where mortality rates were the highest, showed significantly

446 lower photochemical efficiencies than those growing in the three woody microhabitats.

447 These results suggest that both shrubs species enhanced seedling survival by buffering

448 stressful conditions (Smit et al. 2008).

449

450 *Specific combinations of shrub species and life-stages can influence recruitment*

451 *patterns*

452 Our results confirm the hypothesis that the two distinct types of shrub with contrasting

453 characteristics can affect the different stages of the recruitment process differently.

454 Puerta-Piñero et al. (2010) highlighted that differences in disperser activity among

455 vegetation types may modulate holm oak seedling establishment. For instance, the

456 differences in the population physiognomy; *Cistus* forms more clumped populations (ca.

457 80% of cover) than *Retama* (ca. 35% of cover) (Appendix S1), would offer better

458 protection for disperser activity (Muñoz et al. 2009; Pons & Pausas 2007) and could

459 lead to a higher chance of seedling establishment. Nevertheless, we observed that shrub

460 cover effect did not differ between shrub formations (Table 2) and both shrub species

461 showed similar positive effects on seedling emergence (Table 1). These evidences

462 suggest that even low shrub cover formation, as *Retama*, may constituted and effective

463 threshold to enhance seedling emergence. We caution that further investigation relating

464 shrub cover and the factors that determine pre-dispersal and post-dispersal losses, such

465 as disperser activity or acorn predation rates in both vegetation types, is needed to

466 corroborate these observations (Broncano et al. 1998; Cierjacks & Hensen 2004; Perea

467 et al. 2011; Puerta-Piñero et al. 2010; Santos & Tellería 1997).

468

469 Additionally, there must be other factors in *Retama* sites that contribute to losses after

470 seedling emergence compared to that of *Cistus* sites. Results of the acorn sowing

471 experiment showed a higher capacity of *Retama* to improve seedling survival (Fig. 2)

19

472   and morphology (Table 3B) than *Cistus*. However, results of the large-scale survey

473   showed that the survival of seedlings under *Retama* at the end of the summer was

474   similar than under *Cistus* (Table 1). The positive effect of *Retama* on the production of

475   pasture understory (Pugnaire et al. 1996; Rodríguez-Echeverría & Pérez-Fernández

476   2003; Armas et al. 2011; Rivest et al. 2011) suggest that this shrub could attract

477   herbivores and thus may involve higher losses by browsers once seedlings are

478   established. Additionally, increased pasture growth could reinforce competition for soil

479   resources with negative consequences for young seedlings (Cubera et al 2012). By

480   contrast, *Cistus* arrests pasture growth (Rivest et al. 2011) and herbivores may be

481   attracted in a lesser extent. In the acorn sowing experiment, the exclusion cages avoided

482   the activity of browsers, showing, therefore, similar patterns for seedling survival under

483   both types of shrubs.

484

485   Once seedlings have passed the first summer, the outcome that functional traits of the

486   facilitator species exert on the abiotic conditions could be more determining than the

487   benefit of physical protection. Our results showed that the effect of *Retama* was more

488   evident at the sapling stages compared to open spaces than that of *Cistus* (Fig 1). In

489   addition, large sapling abundance in *Retama* formations was much more responsive to

490   small sapling abundance compared to that of *Cistus* formations (Table 2), suggesting an

491   accumulative effect under *Retama*. Smit and Verwijmeren (2011) reported similar

492   results in a grazed wood-pasture system with presence of blackthorn, arguing that a

493   cumulative grazing effect can lead to a strong spatial association between saplings and

494   shrubs over time. The associational resistance theory states that unpalatable species

495   (spiny or toxic) can facilitates palatable species by protecting against herbivores (Baraza

496   et al. 2006; Olff et al. 1999). However, *Retama* lacks of thorns and, as we argued

20

497 previously, its protective effect might be limited, compared to *Cistus* populations. Thus,

498 this positive association between *Retama* and saplings over time might be related to

499 other factors such as improvement of soil conditions (water, Prieto et al. 2010, and

500 nutrients availability, Pugnaire et al. 1996; Rolo et al. 2012) or less competitive effects

501 on tree overstory (Rolo & Moreno 2011).

502

503 Regarding the effect of *Cistus* on saplings, the opposite trend was observed in *Cistus*

504 sites compared to that of *Retama*. Here this effect did not change along life-stages

505 (Table 1). These results might be related to the effect of *Cistus* on soil resources

506 availability. Previous studies have shown that *Cistus* exerts a sharp negative effect on

507 tree overstory functioning (Rolo & Moreno 2011) and productivity (Rivest et al. 2011),

508 probably due to a high competition for soil resources (Rolo et al. 2012). The significant

509 reduction of soil water content that we found under *Cistus* compared to open zones

510 (Table 3A) supports this argument. Both findings of *Cistus* and *Retama* sites are

511 consistent with other studies that have shown how the balance of interactions between

512 nurse and protégé may shift in function of life-stage of one of the species involved

513 (Schupp 1992, 1995; Barnes & Archer 1999; Sthultz et al. 2007).

514

515 **Conclusions**

516 It is known that shrub presence in grazed oak woodlands can play a crucial role for

517 favoring tree recruitment. Here, we have argued that two different shrub species can

518 impose a specific template that is able to affect the whole recruitment process and, thus,

519 the dynamics of the system. Although both shrub species showed similar facilitative

520 patterns to buffer stressful conditions at the seedling stage, the net outcome is the result

521 of different processes that affect specifically each shrub formation. As a result, *Cistus*

522   formations seem to be less effective to guarantee long-term persistence of surviving

523   seedlings than *Retama* formations. Thus, at the long-term the presence of *Cistus* may

524   add further limitations for effective tree recruitment and its populations could

525   perpetuates as a stable state. Our study was not designed to disentangle the effect of the

526   different factors involved in the regeneration process. Rather it assessed the recruitment

527   dynamics in sites encroached with contrasting shrub species. It demonstrated that to

528   plan effective regeneration strategies it is crucial to take into account that different

529   "nurse plants" may have diverging influences on the long-term dynamics of the system.

530   However, further analyses are needed to disentangle the effect of the different factors

531   involved in the regeneration process.

532

539

540   **References**

541   Acacio, V., Holmgren, M., Jansen, P., Schrotter, O. 2007. Multiple recruitment
542   limitation causes arrested succession in Mediterranean cork oak systems.
543   *Ecosystems* 10: 1220-1230.
544   Armas, C., Rodríguez-Echeverría, S., Pugnaire, F.I. 2011. A field test of the stress-
545   gradient hypothesis along an aridity gradient. *Journal of Vegetation Science* 22:
546   818-827.
547   Baraza, E., Zamora, R., Hódar, J.A. 2006. Conditional outcomes in plant–herbivore
548   interactions: neighbours matter. *Oikos* 113: 148-156.
549   Barnes, P.W., Archer, S. 1999. Tree-shrub interaction in a subtropical savanna parkland,
550   competition or facilitation? *Journal of Vegetation Science* 10: 525-536.

551   Bates, D., Maechler, M. 2010. lme4. Linear mixed-effects models using S4 classes.
552   http://lme4.r-forge.r-project.org/

553   Bergmeier, E., Petermann, J., Schröder, E. 2010. Geobotanical survey of wood-pasture
554   habitats in Europe: diversity, threats and conservation. *Biodiversity and*
555   *Conservation* 19: 2995-3014.

556   Blanco, E., Casado, M., Costa, M., Escribano, R., García, M., Génova, M., Gómez, A.,
557   Gómez, F., Moreno, J., Morla, C. 1997. Los bosques ibéricos: una interpretación
558   geobotánica. Planeta.

559   Blondel, J., Aronson, J., Bodiou, J.Y. 2010. The Mediterranean region: biological
560   diversity in space and time. Oxford University Press.

561   Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H.,
562   White,JD.S. 2009. Generalized linear mixed models: a practical guide for
563   ecology and evolution. *Trends in Ecology & Evolution* 24: 127-135.

564   Broncano, M., Riba, M., Retana, J. 1998. Seed germination and seedling performance of
565   two Mediterranean tree especies, holm oak (*Quercus ilex* L.) and Aleppo pine
566   (*Pinus halepensis* Mill.): a multifactor experimental approach. *Plant Ecology*
567   138: 17-26.

568   Brooker, R.W., Maestre, F.T., Callaway, R.M., Lortie, C.L., Cavieres, L.A., Kunstler,
569   G., Liancourt, P., Tielbörger, K., Travis, J.M.J., Anthelme, F., Armas, C., Coll,
570   L., Corcket, E., Delzon, S., Forey, E., Kikvidze, Z., Olofsson, J., Pugnaire, F.,
571   Quiroz, C.L., Saccone, P., Schiffers, K., Seifan, M., Touzard, B., Michalet, R.
572   2008. Facilitation in plant communities: The past, the present, and the future.
573   *Journal of Ecology* 96: 18-34.

574   Cierjacks, A., Hensen, I. 2004. Variation of stand structure and regeneration of
575   Mediterranean holm oak along a grazing intensity gradient. *Plant Ecology* 173:
576   215–223.

577   Cubera, E., Moreno, G. 2007. Effect of single *Quercus ilex* trees upon spatial and
578   seasonal changes in soil water content in dehesas of central western Spain.
579   *Annals of Forest Science* 64: 355-364.

580   Cubera, E., Moreno, G., Solla, A., Madeira, M. 2012. Root system of Quercus suber L.
581   seedlings in response to herbaceous competition and different watering and
582   fertilisation regimes. *Agroforestry Systems*. doi:10.1007/s10457-012-9492-x

583   Díaz, M., Campos, P., Pulido, F. 1997. The Spanish dehesas: a diversity in land-use and
584   wildlife. In: Pain, D. Pienkowski, M. (eds.) *Farming and Birds in Europe. The*
585   *Common Agricultural Policy and its Implications for Bird Conservation.* pp.
586   178-209. Academic Press, London.

587   Eldridge, D.J., Bowker, M.A., Maestre, F.T., Roger, E., Reynolds, J.F., Whitford, W.G.
588   2011. Impacts of shrub encroachment on ecosystem structure and functioning:
589   towards a global synthesis. *Ecology Letters* 14: 709-722.

590   Gea-Izquierdo, G., Allen-Díaz, B., San Miguel, A., Cañellas, I. 2010. How do trees
591   affect spatio-temporal heterogeneity of nutrient cycling in Mediterranean annual
592   grasslands? *Annals of Forest Science* 67: 112-122.

593   Gibbons, P., Lindenmayer, D., Fischer, J., Manning, A., Weinberg, A., Seddon, J.,
594   Ryan, P., Barret, G. 2008. The future of scattered trees in agricultural
595   landscapes. *Conservation Biology* 22: 1309-1319.

596   Gómez, J.M. 2003. Spatial patterns in long-distance dispersal of *Quercus ilex* acorns by
597   jays in a heterogeneous landscape. *Ecography* 26: 573–584.

598 Gómez, J.M. 2004. Importance of microhabitat and acorn burial on *Quercus ilex* early
599   recruitment: non-additive effects on multiple demographic processes. *Plant
600   Ecology* 172: 287-297.
601 Gómez, J.M., Puerta-Piñero, C., Schupp, E.W. 2008. Effectiveness of rodents as local
602   seed dispersers of Holm oaks. *Oecologia* 155: 529–537.
603 Gómez-Aparicio, L. 2008. Spatial patterns of recruitment in Mediterranean plant
604   species: linking the fate of seeds, seedlings and saplings in heterogeneous
605   landscapes at different scales. *Journal of Ecology* 96: 1128–1140.
606 Gómez-Aparicio, L. 2009. The role of plant interaction in the restoration of degraded
607   ecosystems: a meta-analysis across life-forms and ecosystems. *Journal of
608   Ecology* 97: 1202-1214.
609 Gómez-Aparicio, L., Gómez, J.M., Zamora, R., Boettingerm, J. 2005. Canopy vs. soil
610   effects of shrubs facilitating tree seedlings in Mediterranean montane
611   ecosystems. *Journal of Vegetation Science* 16: 191-198.
612 Gómez-Aparicio, L., Zamora, R., Gómez, J., Castro, J., Baraza, E. 2004. Applying plant
613   facilitation to forest restoration: a meta-analysis of the use of shrubs as nurse
614   plants. *Ecological Applications* 14: 1128-1138.
615 Grove, A.T., Rackham, O. 2003. The nature of Mediterranean Europe: an ecological
616   history. Yale Univ Pr.
617 Hoffmann, W.A., Marchin, R.M., Abit, P., Lau, O.L. 2011. Hydraulic failure and tree
618   dieback are associated with high wood density in a temperate forest under
619   extreme drought. *Global Change Biology* 17: 2731-2742.
620 Hothorn, T., Bretz, F., Westfall, P., Heiberger, R., Schuetzenmeister, A. 2010.
621   multcomp. Simultaneous inference in general parametric models. http://cran.r-
622   project.org/web/packages/multcomp/
623 Howlett, D.S., Moreno, G., Losada, M.R.., Nair, P.K.., Nair, V.D. 2011. Soil carbon
624   storage as influenced by tree cover in the Dehesa cork oak silvopasture of
625   central-western Spain. *Journal of Environmental Monitoring* 13: 1897–1904.
626 IUSS Working Group WRB 2006. World Reference Base for Soil Resources, *World
627   Soil Resources Reports*. FAO, Rome.
628 Kollmann, J. 2000. Dispersal of fleshy-fruited species: a matter of spatial scale?
629   *Perspectives in Plant Ecology, Evolution and Systematics* 3: 29–51.
630 Kulmatiski, A., Beard, K.H., Stevens, J.R., Cobbold, S.M. 2008. Plant–soil feedbacks: a
631   meta-analytical review. *Ecology Letters* 11: 980–992.
632 Laborde, J., Guevara, S., Sánchez-Ríos, G. 2008. Tree and shrub seed dispersal in
633   pastures: The importance of rainforest trees outside forest fragments. *Ecoscience
634   15: 6-16.
635 Manning, A., Fischer, J., Lindnmayer, D. 2006. Scattered trees are keystone structures -
636   Implications for conservation. *Biological Conservation* 132: 311-321.
637 Marañón, T., Pugnaire, F., Callaway, R.M. 2009. Mediterranean-climate oak savannas:
638   the interplay between abiotic environment and species interaction. *Web Ecology*
639   9: 30-43.
640 McCreary, D. 2004. Managing and restoring California's oak woodlands. *Natural Areas
641   Journal* 24: 269–275.
642 Moreno, G., Pulido, F.J. 2009. The functioning, management and persistence of
643   Dehesas. In: Rigueiro-Rodriguez, A. McAdam, J. Mosquera-Losada, M.R. (eds.)
644   *Agroforestry in Europe, Current Status and Future Prospects*, *Advances in
645   Agroforestry*. pp. 127-160. Springer, Heidelberg.

Mountford, E.P., Peterken, G.F. 2003. Long-term change and implications for the management of wood-pastures: Experience over 40 years from Denny Wood, New Forest. *Forestry* 76: 19-43.

Muñoz, A., Bonal, R. 2007. Rodents change acorn dispersal behaviour in response to ungulate presence. *Oikos* 116: 1631-1638.

Muñoz, A., Bonal, R., Díaz, M. 2009. Ungulates, rodents, shrubs: interactions in a diverse Mediterranean ecosystem. *Basic Applied Ecology* 10: 151-160.

Olea, L., San Miguel, A. 2006. The Spanish dehesa: a traditional Mediterranean sylvopastoral system linking production and nature conservation. *Grassland Science Europe* 11: 3-13.

Olff, H., Vera, F., Bokdam, J., Bakker, E., Gleichman, J., Maeyer, K., Smit, R. 1999. Shifting mosaics in grazed woodlands driven by the alternation of plant facilitation and competition. *Plant Biology* 1: 127–137.

den Ouden, J., Jansen, P.A., Smit, R. 2005. Jays, mice and oaks: predation and dispersal of *Quercus robur* and *Q. petraea* in North-western Europe. In: Forget, P.M. Lambert, J.E. Vander Wall, S.B. (eds.) *Seed Fate: Predation, Dispersal and Seedling Establishment*. pp. 223–240. CABI Publishing, Wallingford, UK.

Padilla, F.M., Pugnaire, F.I. 2006. The role of nurse plants in the restoration of degraded environments. *Frontiers in Ecology and the Environment* 4: 196–202.

Pausas, J.G., Ribeiro, E., Dias, S.G., Pons, J., Beseler, C. 2006. Regeneration of a marginal *Quercus suber* forest in the eastern Iberian Peninsula. *Journal of Vegetation Science* 17: 729–738.

Perea, R., San Miguel, A., Gil, L. 2011. Acorn dispersal by rodents: the importance to redispersal and distance to shelter. *Basic Applied Ecology in press*.

Plieninger, T., Pulido, F., Konold, W. 2003. Effects of land use history on size structure of Holm oak stands in Spanish dehesas: implication for conservation and restoration. *Environmental Conservation* 30: 61-70.

Plieninger, T., Rolo, V., Moreno, G. 2010. Large-scale patterns of *Quercus ilex*, *Quercus suber* and *Quercus pyrenaica* regeneration in Central-Western Spain. *Ecosystems* 13: 644-660.

Plieninger, T., Schaich, H., Kizos, T. 2011. Land-use legacies in the forest structure of silvopastoral oak woodlands in the Eastern Mediterranean. *Regional Environmental Change* 11: 603-615.

Plieninger, T., Wilbrand, C. 2001. Land use, biodiversity conservation, and rural development in the dehesas of Cuatro Lugares, Spain. *Agroforestry Systems* 51: 23–34.

Pons, J., Pausas, J.G. 2007. Rodent acorn selection in a Mediterranean oak landscape. *Ecological Research* 22: 535–541.

Prieto, I., Kikvidze, Z., Pugnaire, F. 2010. Hydraulic lift: soil processes and transpiration in the Mediterranean leguminous shrub *Retama sphaerocarpa* (L.) Boiss. *Plant and Soil* 329: 447-456.

Puerta-Piñero, C., Gómez, J.M., Valladares, F. 2007. Irradiance and oak seedling survival and growth in a heterogeneous environment. *Forest Ecology and Management* 242: 462-469.

Puerta-Piñero, C. 2010. Intermediate spatial variations on acorn predation shapes Holm oak establishment within a Mediterranean landscape context. *Plant Ecology* 210: 213–224.

Pugnaire, F., Haase, P., Puigdefábregas, J. 1996. Facilitation between higher plant species in a semi-arid environment. *Ecology* 77: 1420-1426.

Pugnaire, F.I., Armas, C., Maestre, F.T. 2011. Positive plant interactions in the Iberian Southeast: Mechanisms, environmental gradients, and ecosystem function. *Journal of Arid Environments* 75: 1310-1320.

Pulido, F., Díaz, M. 2005. Regeneration of a Mediterranean oak: a whole cycle approach. *EcoScience* 12: 92-102.

Pulido, F., Díaz, M., Hidalgo, S. 2001. Size-structure and regeneration of Spanish holm oak Quercus ilex forest and dehesas: effects of agroforestry use on their long-term sustainability. *Forest Ecology and Management* 146: 1-13.

Pulido, F., García, E., Obrador, J., Moreno, G. 2010. Multiple pathways for tree regeneration in anthropogenic savannas: incorporating biotic and abiotic drivers into management schemes. *Journal of Applied Ecology* 47: 1272-1281.

Ramírez, J., Díaz, M. 2008. The role of temporal shrub encroachment for the maintenance of Spanish holm oak *Quercus ilex* dehesas. *Forest Ecology and Management* 255: 1976-1983.

R Development Core Team 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Rivest, D., Rolo, V., López-Díaz, L., Moreno, G. 2011. Shrub encroachment in Mediterranean silvopastoral systems: *Retama sphaerocarpa* and *Cistus ladanifer* induce contrasting effects on pasture and *Quercus ilex* production. *Agriculture, Ecosystems & Environment* 141: 447-454.

Rodríguez-Calcerrada, J., Cano, F.J., Valbuena-Carabaña, M., Gil, L., Aranda, I. 2010. Functional performance of oak seedlings naturally regenerated across microhabitats of distinct overstorey canopy closure. *New Forests* 39: 245-259.

Rodríguez-Echeverría, S., Pérez-Fernández, M. 2003. Soil fertility and herb facilitation mediated by *Retama sphaerocarpa*. *Journal of Vegetation Science* 14: 807-814.

Rolo, V., Moreno, G. 2011. Shrub species affect distinctively the functioning of scattered *Quercus ilex* trees in Mediterranean open woodlands. *Forest Ecology and Management* 261:1750-1759.

Rolo, V., López-Díaz, M., Moreno, G. 2012. Shrubs affect soil nutrients availability with contrasting consequences for pasture understory and tree overstory production and nutrient status in Mediterranean grazed open woodlands. *Nutrient Cycling in Agroecosystems* 93: 89-102.

Salehi, A., Wilhelmsson, E., Söderberg, U. 2008. Land cover changes in a forested watershed, southern Zagros, Iran. *Land Degradation & Development* 19: 542–553.

Santos, T., Tellería, J.L. 1997. Vertebrate predation on Holm Oak, *Quercus ilex*, acorns in a fragmented habitat: effects on seedling recruitment. *Forest Ecology and Management* 98: 181–187.

Schupp, E.W. 1992. The Janzen-Connell model for tropical tree diversity: population implications and the importance of spatial scale. *The American Naturalist* 140: 526–530.

Schupp, E.W. 1995. Seed-seedling conflicts, habitat choice, and patterns of plant recruitment. *American Journal of Botany* 399–409.

Steenkamp, C.J., Vogel, J.C., Fuls, A., van Rooyen, N., van Rooyen, M.W. 2008. Age determination of *Acacia erioloba* trees in the Kalahari. *Journal of Arid Environments* 72: 302-313.

Silva, J.S., Rego, F.C. 2004. Root to shoot relationships in Mediterranean woody plants from Central Portugal. *Biologia* 59: 109–115.

744 Smit, C., den Ouden, J., Díaz, M. 2008. Facilitation of *Quercus ilex* recruitment by
745 shrubs in Mediterranean open woodlands. *Journal of Vegetation Science* 19:
746 193-200.
747 Smit, C., Den Ouden, J., Müller-Schärer, H. 2006. Unpalatable plants facilitate tree
748 sapling survival in wooded pastures. *Journal of Applied Ecology* 43: 305-312.
749 Smit, C., Díaz, M., Jansen, P. 2009. Establishment limitation of holm oak (Quercus ilex
750 subsp. ballota (Desf.) Samp.) in a Mediterranean savanna-forest ecosystem.
751 *Annals of Forest Science* 66: 511.
752 Smit, C., Verwijmeren, M. 2011. Tree-shrub associations in grazed woodlands: first
753 rodents, then cattle? *Plant Ecology* 212: 483-493.
754 Sosa, T., Valares, C., Alías, J., Chaves, N. 2010. Persistence of flavonoids in *Cistus
755 ladanifer* soils. *Plant and Soil* 337: 51-63.
756 Sthultz, C.M., Gehring, C.A., Whitham, T.G. 2007. Shifts from competition to
757 facilitation between a foundation tree and a pioneer shrub across spatial and
758 temporal scales in a semiarid woodland. *New Phytologist* 173: 135–145.
759 Terradas, J. 1999. Holm Oak and Holm Oak Forests: An introduction. In: Rodá, F.
760 Retana, J. Gracia, C.A. Bellot, J. (eds.) *Ecology of Mediterranean Evergreen
761 Oak Forest.*, *Ecological Studies*. pp. 145-144. Springer, Berlin.
762 Trichon, V., Walter, J.M.., Laumonier, Y. 1998. Identifying spatial patterns in the
763 tropical rain forest structure using hemispherical photographs. *Plant Ecology*
764 137: 227–244.
765 Tyler, C., Kuhn, B., Davis, F. 2006. Demography and recruitment limitations of three
766 oak species in California. *Quarterly Review of Biology* 81: 127-152.
767 Valladares, F., Guzmán, B. 2006. Canopy structure and spatial heterogeneity of
768 understory light in an abandoned Holm Oak woodland. *Annals of Forest Science*
769 63: 749-761.
770 Vander Wall, S.B. 2001. The evolutionary ecology of nut dispersal. *The Botanical
771 Review* 67: 74-117.
772 Weinberg, A., Gibbons, P., Briggs, S.V., Bonser, S.P. 2011. The extent and pattern of
773 *Eucalyptus* regeneration in an agricultural landscape. *Biological Conservation*
774 144: 227-233.
775 Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M. 2009. Mixed effects
776 models and extensions in ecology with R. Springer Verlag.
777
778 **Supporting Information**
779 Additional supporting information may be found in the online version of this article:
780
781 Appendix S1. Main characteristics of tree and shrub layers of surveyed sites encroached
782 either with *Cistus* or *Retama*
783

784   Table 1 Results of GLMMs from the survey data to test the effect of type of site (*Cistus* or
785   *Retama*) and microhabitat (open spaces, tree, shrub and tree-shrub) on number of emerged and
786   survived seedling and small and large sampling. Site and year were considered random effects.
787

	Emerged Seedling			Survived Seedling			Small Sapling			Large Sapling		
	χ^2	d.f.	*P* value	χ^2	d.f.	*P* value	χ^2	d.f.	*P* value	χ^2	d.f.	*P* value
Type (T)	6.9	1	**0.004**	6.4	1	**0.011**	851.7	1	**<0.001**	0.1	1	0.716
Microhabitat (M)	140.7	3	**<0.001**	275.3	3	**<0.001**	68.6	3	**<0.001**	55.2	3	**<0.001**
T x M	2.3	3	0.517	1.9	3	0.599	12.0	3	**0.007**	33.6	3	**<0.001**

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792 Table 2 Results of GLMMs from the survey data to test the effect of type of site (*Cistus* or
793 *Retama*), tree diameter at breast height (TD), shrub cover (SC), time after abandonment (TA)
794 and density of previous regeneration stage (DPS), on number of emerged and survived seedling
795 and small and large sampling in the encroached plots. Site and year were considered random
796 effects.
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|	Emerged Seedlings			Survived Seedling			Small Saplings			Large Saplings		
	χ^2	d.f	*P* value	χ^2	d.f	*P* value	χ^2	d.f	*P* value	χ^2	d.f	*P* value
Type (T)	7.4	1	**0.007**	8.7	1	**0.003**	10.4	1	**0.001**	0.1	1	0.879
Tree DBH (TD)	4.5	1	**0.034**	0.1	1	0.886	2.4	1	0.124	3.9	1	**0.048**
Shrub Cover (SC)	0.9	1	0.348	0.2	1	0.305	1.3	1	0.249	0.2	1	0.691
Time after abandonment (TA)	1.1	1	0.292	1.1	1	0.305	3.7	1	*0.053*	2.4	1	0.121
Density Prev. Stage (DPS)	-	-	-	4.1	1	**0.042**	33.3	1	**< 0.001**	3.5	1	*0.062*
T x SC	0.2	1	0.666	0.1	1	0.999	0.7	1	0.398	0.2	1	0.659
T x TA	1.8	1	0.176	0.8	1	0.383	1.2	1	0.276	0.3	1	0.579
T x DPS	-	-	-	0.6	1	0.449	1.0	1	0.323	4.3	1	**0.039**

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Table 3 Averaged microclimatic characteristics (A) and comparison of morphological and functional parameters (B) of surviving seedlings from the acorn sowing experiment under shrub, tree, tree-shrubs and open spaces in sites encroached with *Cistus* or *Retama*. Different letters between habitats denote significant differences at $P < 0.05$.

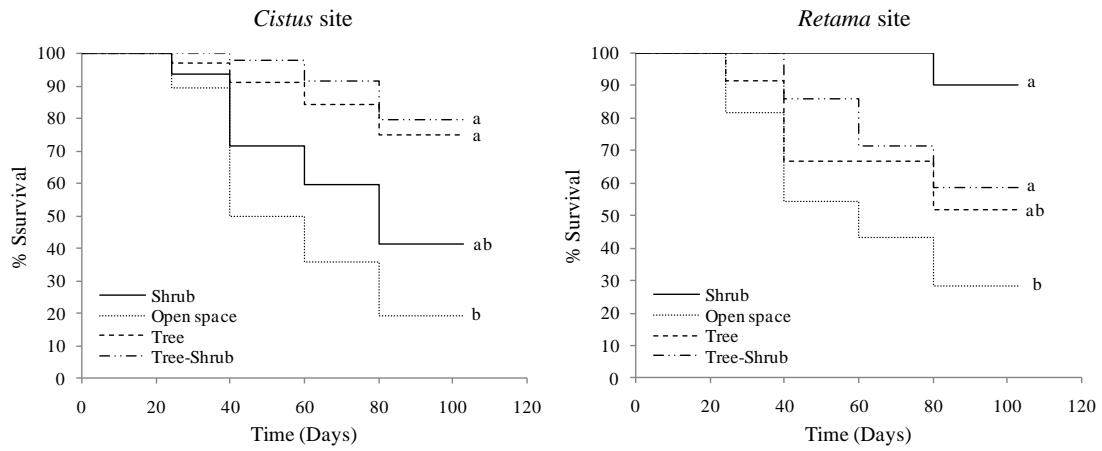| | *Cistus* site | | | | Microhabitat Effect | |
A. Microclimate	Shrub	Tree-shrub	Open spaces	Tree	F_{df-n}	P-value
Temp., ºC	28.3 ± 0.2a	27.4 ± 0.14b	28.43 ± 0.19a	27.57 ± 0.14b	10.0 $_{3-8680}$	**<0.001**
VPD, kPa	2.4 ± 0.03a	2.1 ± 0.03b	2.40 ± 0.04a	2.11 ± 0.03b	27.4 $_{3-8680}$	**<0.001**
Irradiance, %	45.0 ± 1.6ab	39.8 ± 2.45b	76.27 ± 2.02a	33.23 ± 3.67b	17.0 $_{3-24}$	**<0.001**
Soil moisture, %	18.9 ± 1.0c	22.2 ± 0.98a	22.16 ± 0.98ab	21.11 ± 0.98bc	2.4 $_{3-60}$	*0.073*
B. Morphology and performance						
Height (cm)	5.95 ± 0.24	5.91 ± 0.28	5.88 ± 0.45	5.55 ± 0.2	0.65 $_{3-221}$	0.581
Stem density	0.63 ± 0.03	0.66 ± 0.04	0.73 ± 0.04	0.68 ± 0.02	1.2 $_{3-40}$	0.319
SLA (cm²/g)	212.7 ± 8.8a	222.8 ± 14.7a	175 ± 6.7b	201.4 ± 7.0a	3.8 $_{3-40}$	**0.017**
LAR (cm²/g)	149.7 ± 13.6	152.9 ± 15.0	129.8 ± 7.4	151.2 ± 9.6	0.9 $_{3-40}$	0.441
F_v/F_m	0.80 ± 0.01a	0.79 ± 0.01ab	0.75 ± 0.01c	0.78 ± 0.01b	18.1 $_{3-229}$	**<0.001**
	Retama site				Microhabitat Effect	
A. Microclimate	Shrub	Tree-shrub	Open spaces	Tree	F_{df-n}	P-value
Temp., ºC	25.73 ± 0.24b	26.11 ± 0.23b	26.91 ± 0.23a	26.10 ± 0.3b	6.3 $_{3-5944}$	**0.002**
VPD, kPa	2.17 ± 0.05	2.22 ± 0.04	2.28 ± 0.04	2.08 ± 0.04	1.6 $_{3-5944}$	0.208
Irradiance, %	58.23 ± 4.04ab	44.08 ± 2.55b	84.37 ± 2.98a	45.08 ± 4.07b	16.2 $_{3-24}$	**0.001**
Soil moisture, %	20.49 ± 0.96	20.03 ± 0.96	19.71 ± 0.96	19.62 ± 0.96	0.17 $_{3-60}$	0.918
B. Morphology and performance						
Height (cm)	7.70 ± 0.83a	5.83 ± 0.58b	5.62 ± 0.3b	4.1 ± 0.6c	3.6 $_{3-246}$	**0.014**
Stem density	0.66 ± 0.03b	0.73 ± 0.03a	0.73 ± 0.02a	0.67 ± 0.03ab	2.3 $_{3-40}$	*0.089*
SLA (cm²/g)	199.5 ± 10.6a	187.1 ± 8.3b	157.6 ± 2.6b	193.6 ± 7.0b	4.4 $_{3-40}$	**0.009**
LAR (cm²/g)	151.5 ± 5.7a	142.2 ± 9.9ab	120.1 ± 2.8b	150.1 ± 8.7a	2.7 $_{3-40}$	*0.059*
F_v/F_m	0.76 ± 0.01a	0.79 ± 0.01a	0.75 ± 0.01b	0.77 ± 0.02a	3.1 $_{3-243}$	**0.026**

SLA: calculated as the ratio of leaf area to leaf dry mass
LAR: calculated as the ratio of leaf area to plant dry mass

807   Figure 1 Abundance of emerged and survived seedlings and small and large saplings
808   (Number / 100 m^2) in open spaces, tree, shrub and tree-shrub microhabitats from the
809   survey data in sites encroached with *Cistus* or *Retama.* Different letters between
810   microhabitats within type of site denote significant differences at $P < 0.05$.
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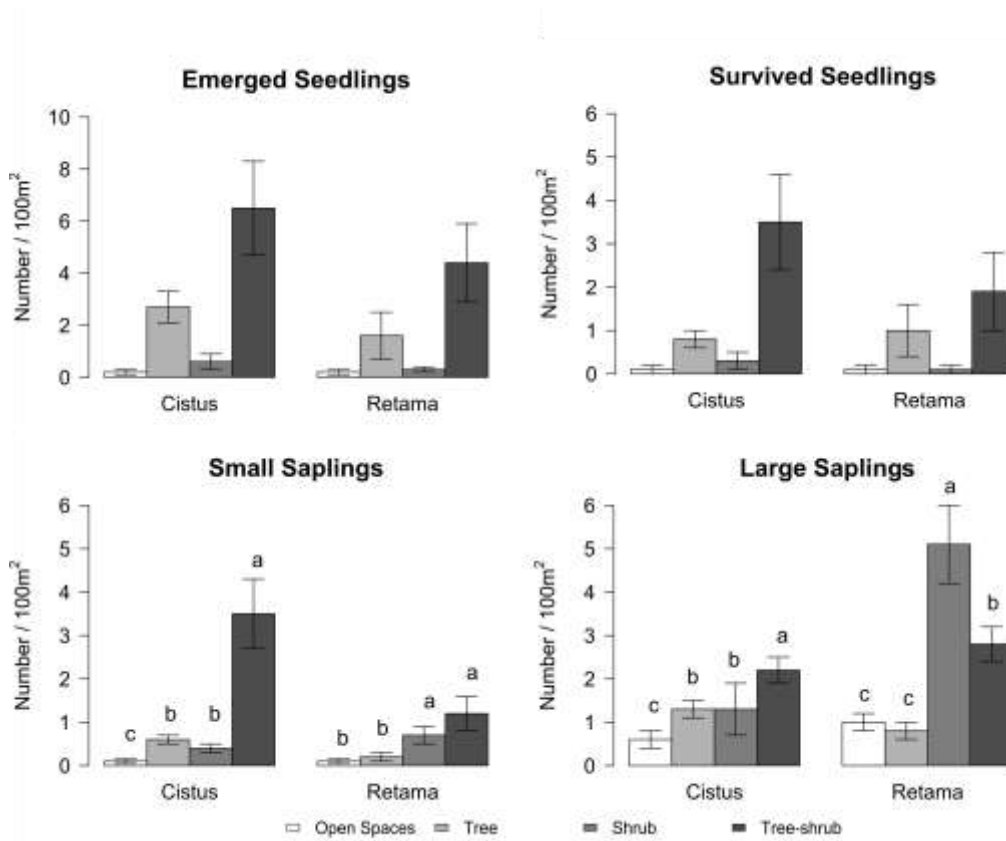
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813 Figure 2. Cumulative survival proportion over summer, from June to September, of
814 seedlings growing under shrub, tree, tree-shrubs and open spaces from the acorn sowing
815 experiment in sites encroached with *Cistus* or *Retama*. Different letters denote
816 significant differences at $P < 0.05$.
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