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8 | **Facilitation of *Quercus ilex* in Mediterranean shrubland is explained by both direct and**
9 | **indirect interactions mediated by herbs**

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22 | **Running headline:** Direct and indirect facilitation in Mediterranean shrubland

23 **Summary**

- 24 1. Competitive and facilitative interactions shape plant communities. Whereas a number of
25 studies have addressed competition and direct facilitation among plants in dry ecosystems,
26 indirect facilitation has received little attention.
- 27 2. We investigated the relative importance of direct and indirect facilitation by the nurse plant
28 *Retama sphaerocarpa* on late-successional *Quercus ilex* seedlings mediated by herb
29 suppression in a Mediterranean shrubland in 2006 and 2007. We also studied whether
30 facilitation outcome depended on the size of the facilitated seedlings.
- 31 3. A field experiment was carried out to test the effect of (1) position of *Q. ilex* seedling with
32 respect to shrub canopy (under shrubs or in gaps), (2) herb competition (presence or
33 absence), and (3) seedling size. 2006 was an average rainfall year while 2007 had a much
34 more humid spring and a dryer summer than 2006.
- 35 4. In both years, nurse shrubs reduced seedling mortality whereas herbs increased it. In the
36 average rainfall year, seedling mortality under shrubs was unaffected by herbs whereas in
37 gaps it was significantly higher in presence of herbs. This showed that the nurse shrub
38 indirectly facilitated the seedlings by reducing the competitive capacity of herbs.
39 Conversely, facilitation was predominately direct during the humid spring and dry summer
40 year since herbs hindered seedling survival similarly under the nurse shrub and in gaps.
41 The nurse shrub directly facilitated the seedlings by reducing seedling photoinhibition and
42 water stress.
- 43 5. Improvement of environmental conditions by *Retama* benefited smaller seedlings but not
44 larger seedlings since the nurse shrub reduced mortality of smaller seedlings relative to
45 that in gaps, but this effect was not observed for larger seedlings. This indicates that
46 individuals within a seedling population may not have the same response to facilitation.
- 47 6. *Synthesis*. Both indirect and direct facilitation are important mechanisms for *Q. ilex*
48 regeneration in *Retama* shrubland and their importance seems to vary with climatic

49 conditions. Indirect facilitation by release of herb competition under nurse shrubs is
50 important in years of dry springs when competition between nurse shrubs and herbs is
51 high, whereas direct facilitation mediated by microclimate amelioration increases with
52 summer aridity.

53

54 **Key-words:** Chlorophyll fluorescence, herb competition, *Quercus ilex*, *Retama sphaerocarpa*,
55 seedling size, water potential

56

57 **Introduction**

58 Biotic interactions are major drivers of the structure and dynamics of plant communities
59 (Lortie *et al.* 2004). Competition has traditionally been considered as the major interaction that
60 structures plant communities (Grime 1974). However, in the last decade, an increasing number
61 of studies have also emphasized the importance of facilitative or positive interactions (Bertness
62 & Callaway 1994; Callaway 1995; Brooker *et al.* 2008). Positive and negative interactions
63 among organisms act simultaneously, and the net balance of these interactions determines the
64 community organization and composition (Callaway & Walker 1997; Holmgren, Scheffer &
65 Huston 1997; Brooker *et al.* 2008). The balance of plant–plant interactions is context-
66 dependent, varying in response to abiotic stress, disturbance, life cycle stage, species identity
67 and interactions with other neighbours (Callaway 2007).

68 Facilitation can be direct, i.e. one species increases the performance of a second species
69 by ameliorating the abiotic environment (Callaway 1995; Pugnaire, Haase & Pugdefábregas
70 1996a; Pugnaire, Armas & Valladares 2004). Facilitation may also be indirect, i.e. a third
71 species mediates interactions between the nurse species and the target species. Thus, some
72 plants protect other plants from herbivores, attract pollinators, concentrate propagules of other
73 species, or enhance mycorrhizae and soil microbial activity (Callaway 1995, 2007). The
74 occurrence of a third plant species may also convert the competition between two species into

75 indirect facilitation via suppression of a shared competitor (Miller 1994; Levine 1999;
76 Callaway & Pennings 2000). Levine (1999) predicted that indirect facilitation mediated by a
77 third competitor would mainly occur in a system in which the three species compete for
78 different resources or use different mechanisms to acquire them. This argument is supported by
79 previous studies (Siemann & Rogers 2003; Kunstler *et al.* 2006).

80 In contrast to direct facilitation and competition, indirect facilitation has received less
81 attention. Most studies assessing indirect facilitation have focused on interactions between
82 different trophic levels, such as those analysing defence against herbivory (Boulant *et al.* 2008;
83 Gómez-Aparicio *et al.* 2008; Anthelme & Michalet 2009) or concentration of propagules of
84 other species (Verdú & García-Fayos 2003; Aerts *et al.* 2006). Experimental field studies
85 investigating indirect facilitation within the same trophic level are much less frequent (Brooker
86 *et al.* 2008). Most studies of indirect facilitation among plants have been performed in
87 productive or moderately productive environments; whereas some of them documented
88 indirect facilitation (Levine 1999; Callaway & Pennings 2000; Siemann & Rogers 2003;
89 Kunstler *et al.* 2006), others failed to detect this process (Pagès & Michalet 2003; Pagès *et al.*
90 2003). To our knowledge, no experimental field study to date has explored the importance of
91 indirect facilitation in semi-arid or arid systems.

92 Direct facilitation is a recognized recruitment mechanism for plants in Mediterranean
93 environments (Verdú & García-Fayos 2003; Gómez-Aparicio, Gómez & Zamora 2005a), but
94 the importance of indirect facilitation remains to be assessed in this system. *Retama*
95 *sphaerocarpa* (*Retama* henceforth) is one of the most-studied Mediterranean nurse species. It
96 promotes the development of a diverse herbaceous community due to changes in microclimate
97 and soil fertility under its canopy (Pugnaire *et al.* 1996b; Pugnaire *et al.* 2004). Although herbs
98 compete with the seedlings of woody species and impair woodland regeneration (Nambiar &
99 Zed 1980; Rey Benayas *et al.* 2005), mid- and late-succession woody species such as *Quercus*
100 *ilex* develop under *Retama* canopy (Tovar 2009). Several studies have demonstrated the direct

101 facilitative effect of *Retama* on the plant community developed under its canopy, but no study
102 has assessed its potential indirect facilitative effect (Pugnaire *et al.* 1996a; Pugnaire *et al.*
103 1996b; Rodríguez-Echeverría & Pérez-Fernández 2003; Pugnaire *et al.* 2004).

104 There is an increasing body of evidence that the response to facilitation and that the
105 facilitating ability of nurse species are species-specific (Gómez-Aparicio *et al.* 2004;
106 Liancourt, Callaway & Michalet 2005; Padilla & Pugnaire 2009). However, to our knowledge,
107 no study has tested whether the response to facilitation varies among individuals of the same
108 species. Individuals of the same species may have distinct functional traits that may determine
109 their performance. For example, *Q. ilex* seedlings produced by different mother trees exhibit
110 significant size differences that influence their performance (Leiva & Fernández-Alés 1998).
111 Seedling size can affect survival, with large seedlings having a greater chance of survival under
112 stressful conditions than small seedlings (Cook 1980; Leishman & Westoby 1994)..

113 In the present study, we addressed the following questions: 1) In addition to direct
114 facilitation, is indirect facilitation mediated by herbs an important mechanism for *Q. ilex*
115 regeneration in *Retama* shrubland? 2) Does the response of *Q. ilex* seedlings to facilitation
116 depend on seedling size? To answer these questions, we conducted a factorial field experiment
117 in two consecutive years. We measured the performance of *Q. ilex* seedlings of contrasting size
118 planted under *Retama* canopy and in gaps, as well as in the presence and absence of herbs.

119

120 **Materials and methods**

121 **STUDY SITE AND SPECIES STUDIED**

122 The study site is located in La Mancha, central Spain (38°38' N, 3°28' W), at an altitude
123 of 714 m a.s.l. The climate is continental Mediterranean, with a mean annual precipitation of
124 370 mm and a mean annual temperature of 15.2 °C. Summers are very hot and dry and last for
125 three to five months, while winters are cold with frequent frosts. The soil is a poorly developed

126 inceptisol with relatively high clay and quartzite content. The experiment was performed on an
127 abandoned flat wheat cropland that was planted with *R. sphaerocarpa*, *Pinus pinea* and *Q. ilex*
128 in 2000. Each of these three species had the same density, and individuals were planted in rows
129 separated 4m and within each row the distance between plants was 3m. Species were randomly
130 distributed within each row. At the start of the experiment, *P. pinea* and *Q. ilex* trees were
131 more than 2 m and 1m high, respectively, while *Retama* shrubs were c. 2 m high. The
132 understorey vegetation was composed of an herbaceous community, which emerges in the
133 autumn, flowers in spring and senesces during early summer. Annuals such as *Avena barbata*,
134 *Bromus* spp., *Hordeum murinum*, *Lolium rigidum*, *Taeniatherum caput-medusae*, *Rapistrum*
135 *rugosum* as well as several *Trifolium* species dominated this community.

136 The nurse species *R. sphaerocarpa* is a leguminous, broom-like deep-rooted shrub that is
137 native to the Iberian Peninsula and north-west Africa, where it forms shrublands that have
138 traditionally been managed for sheep grazing and hunting. In the last decades, this shrub has
139 colonized large areas of abandoned cropland, and it has been used for subsidized plantation
140 programs. The target benefactor species was *Q. ilex*, an evergreen late-successional oak tree
141 native to the western Mediterranean Basin. It constitutes most of the community biomass in
142 areas where it thrives. However, *Q. ilex* seedlings display low survival rates in forest
143 plantations compared with other Mediterranean forest species (Baeza *et al.*, 1991).

144

145 EXPERIMENTAL DESIGN

146 A factorial field experiment was performed with three factors: (1) position with respect
147 to the *Retama* canopy (under or outside), (2) herb abundance (presence or absence) and (3) *Q.*
148 *ilex* seedling size. The experiment was conducted in 2006 and 2007. In 2006, the rainfall and
149 mean temperature during the experimental period (February-September) were 255 mm and
150 19.2 °C, respectively, while in 2007 they were 352 mm and 17.7 °C, respectively. The mean
151 historical rainfall (over a 70-year period) and temperature (over a 55-year period) during the

152 same period were 256 mm and 17.5 °C, respectively. Thus, 2006 can be considered as an
153 average rainfall year and 2007 as a humid year. Differences between years mainly occurred in
154 spring (February to May), which was much more humid in 2007 than in 2006 (150 and 267
155 mm in 2006 and 2007, respectively). However, summer (June to September) was more humid
156 in 2006 than in 2007 (105 and 85 mm in 2006 and 2007, respectively).

157 We randomly selected 30 *Retama* shrubs with an average canopy height and diameter of
158 2 and 2.3 m, respectively, and 30 gaps located 1.5-2 m outside of the shrub canopies, where the
159 ground was not shaded during most of the day. Each *Retama* shrub was separated from its
160 neighbour by at least 3-4 m. The distribution of the experimental *Retama* shrubs and gaps was
161 not paired, *i.e.* a particular *Retama* shrub was not associated with a particular gap in the field.
162 In each position (under shrub and gap), we distinguished two herb abundance levels (presence
163 or absence). In the microsites with absence of herbs, herbaceous vegetation was removed with
164 herbicide in an area of 0.55-0.6 m² (Oxyfluorfen 24%, Inteike, TradeCorp, Madrid, Spain) at
165 the beginning of the experiment in both years. Finally, in each of these four microsites, *Q. ilex*
166 seedlings were planted in 30 cm-deep holes made with a mechanical auger, minimizing the
167 disturbance of the herbaceous community at the microsites with herb presence. Planting was
168 performed in February in both years. The experimental plot was fenced to exclude medium and
169 large herbivores.

170 We planted seedlings that largely varied in size in both years. In 2006, to promote
171 seedling size differences, we varied (1) the seeding date in the nursery, which affected
172 emergence time and, therefore, the length of the growing period (14 months *vs.* 11 months),
173 and (2) we used different nitrogen fertilization rates. In 2007, we did not apply any cultivation
174 treatment and randomly selected the plants from a standard nursery crop that had a high
175 variation in seedling size. In 2006, the 10th and 90th percentiles of plant height were 9 and 22
176 cm, respectively, while in 2007 they were 12 and 28 cm, respectively. The seedlings were

177 cultivated following standard nursery cultivation methods (Villar-Salvador *et al.* 2004). The
178 acorns used to grow seedlings from were of the same provenance in both years.

179

180 MICROCLIMATE, SOIL AND HERB COMMUNITY CHARACTERISTICS

181 To analyse the effect of the nurse shrub on the environmental conditions under its
182 canopy and on the characteristics of the herb community, we measured several abiotic and
183 biotic variables under the *Retama* canopy and in the gaps. Incident photosynthetic photon flux
184 density at seedlings' mid-height was measured using a quantum photo/radiometer (HD 9021,
185 Delta OHM, Casselle Di Selvazzano, Italy) for all of the planted seedlings. Measurements were
186 carried out in May of both years on clear days between 12:00 and 14:00 h solar time. Seedling
187 leaf temperature and the relative humidity and the temperature in the air were measured in
188 spring of 2006 under the canopy of nine shrubs and in nine gaps. The latter were measured in
189 both presence and absence of herbs with a thermo-hygrometer (HI 9065, HANNA Instruments,
190 Eibar, Spain). The seedling leaf temperature was only measured in the microsites with herbs
191 using a thermocouple inserted under a dry average-sized oak leaf, which was held horizontally
192 during the measurements.

193 Soil samples excluding litter and stones were collected 5-10 cm deep under the canopy
194 of nine shrubs and in nine gaps for nutrient analyses. After air-drying, the samples were sieved
195 through a 2-mm sieve and finely ground. The organic matter was analysed using the method
196 proposed by Walkley & Black (1934). Total N concentration was determined by Kjeldahl
197 analysis with SeSO₄-K₂SO₄ as the catalyst, while the K concentration was measured with an
198 Optic PLASMA ICP (Perkin-Elmer, model 4300 DV, Waltham, Massachusetts, USA),
199 according to MAPA (1986). Soil pH was determined for a 1:2.5 mass-to-volume soil and water
200 suspension. Soil temperature at a 5 cm depth was recorded with a digital thermometer in May
201 2006 in both presence and absence of herbs under nine *Retama* shrubs and in nine gaps.
202 Finally, soil water content in a 0-30 cm soil profile was measured in all planted seedlings in

203 late spring (25 May 2006 and 30 May 2007) and in mid-summer (18 July 2006 and 15 July
204 2007) using Time Domain Reflectometry (TDR, Tektronix, Beaverton, CO, USA) according
205 to Cassel *et al.* (1994). Two 35 cm long stainless-steel rods were inserted one month after
206 planting at a 5-10 cm distance from plants.

207 In late May of both years, when flowering of the herbaceous community was ending, we
208 visually recorded the percentage of herb cover around each *Q. ilex* seedling, as well as the
209 specific composition and abundance of herbs under nine nurse shrubs and in nine gaps using a
210 50 × 50 cm quadrat. Herb species were classified as graminoids and non-graminoids. In early
211 June, when the herbaceous community started to wither, the above-ground herb biomass
212 contained in a 50 × 50 cm quadrat was measured under nine *Retama* shrubs and in nine gaps
213 after drying at 50 °C for two days. The *Retama* shrubs and gaps used to measure the relative air
214 humidity and the air, leaf and soil temperature were randomly selected among all shrubs under
215 which *Q. ilex* seedlings were planted, whereas the *Retama* shrubs and gaps used to determine
216 the soil nutrient concentration and herb mass were randomly selected among shrubs and gaps
217 where no seedlings were planted.

218

219 *QUERCUS ILEX* SEEDLING PERFORMANCE

220 The mortality of *Q. ilex* seedlings was recorded at the end of spring (last week of May)
221 and at the end of the summer (last week of September) in both years. We measured the spring
222 (February to June), summer (June to September) and all first growing season (February to
223 September) seedling mortality. Seedling growth was measured as the stem volume increase and
224 calculated as the difference between the seedling stem volume at the end of the summer and at
225 the planting date. Stem volume was calculated from the height and diameter of the stem,
226 assuming a cone-shaped stem. The diameter was measured immediately above the cotyledon
227 insertion points. We measured the maximum photochemical efficiency of photosystem II

228 (F_v/F_m) and the leaf water potential (ψ) in mid summer of both years (7 July 2006 and 2 July
229 2007) in 3-8 seedlings per treatment to assess the physiological performance of oak seedlings.
230 The F_v/F_m ratio is a good indicator of plant photoinhibition caused by stress factors such as
231 drought, high radiation and frost (Maxwell & Johnson 2000), whereas ψ is a measure of the
232 water status of plants (Flexas *et al.* 2004). F_v/F_m was measured with a portable fluorometer
233 (Hansatech Instruments, Norfolk, England) at predawn and midday (between 12:00 and 14:00
234 h solar time) after 30 minutes of dark adaptation. Leaf water potential was determined at
235 midday using a pressure chamber; it was measured at midday because the value then usually
236 reflects the maximum water stress experienced by the plant within a day. We only measured ψ
237 once a day to prevent distorting the water and carbon economy of seedlings.

238

239 DATA ANALYSIS

240 Differences among microsites in 2006 with regard to air relative humidity and leaf
241 temperature were analysed with two-way ANOVA, where the main factors were position
242 (under *Retama* canopy *vs.* gaps) and herb abundance (absence *vs.* presence of herbs). The
243 differences between the nurse shrub locations and the gaps, both in presence of herbs, in soil
244 nutrient concentration, soil pH and leaf temperature in 2006 were analysed with Student's t-
245 test. Differences in irradiance among treatments were analysed with ANOVA where the main
246 factors were year (2006 *vs.* 2007), position and herb abundance. Soil water content was
247 analysed with repeated-measures ANOVA where between-effects were year and position and
248 the within-effect was season (spring *vs.* summer). Total herbaceous mass was analysed with
249 ANOVA, where the main factors were year and position. Differences in the floristic
250 composition of the herbaceous community were analysed with semi-parametric MANOVA,
251 where dependent variables were the cover of every herb species. Graminoid and non-graminoid

252 covers were analysed with ANOVA in which the main factors were year, position and herb
253 guild (graminoid vs. non-graminoid).

254 Seedling mortality was analysed using a generalized linear model with a binomial
255 distribution and a logit link function that included a herb competition surrogate (see below) and
256 seedling stem volume at the planting date as co-variables. Seedling stem volume was highly
257 correlated with total plant mass ($r=0.92$, $P<0.001$), which was determined in 30 plants
258 randomly harvested from the same lot of plants used for the field experiment in 2006 after
259 removing the growing media and oven drying for 48h. Growth and F_v/F_m in both years were
260 analysed using ANCOVA. The factors in these analyses were year and position; as in the
261 previous analysis, the surrogate of herb competition and seedling stem volume at the planting
262 date were co-variables. We used a herb competition co-variable instead of a qualitative factor
263 (absence vs. presence of herbs) in these analyses because the herb community under *Retama*
264 canopies and in gaps had a different floristic composition and above-ground biomass (Table 1),
265 which can lead to a distinct competition capacity of herbs at each position. The herb
266 competition co-variable values were calculated for each planted seedling in the spring (late
267 May) and summer (mid July) using the first factor scores obtained from a Principal Component
268 Analysis on herb cover, soil volumetric water content and photon flux density around the
269 seedlings. The values of soil water content and irradiance were relative values from field
270 measurements taken under each *Retama* canopy and in each gap; they were calculated as the
271 relative reduction in soil humidity and irradiance in the microsite with herbs with respect to the
272 adjacent microsite without herbs. This removes the effect of the nurse shrub. As the ψ of many
273 of the plants exceeded the measurement limit of the pressure chamber (6.8 MPa) in both years,
274 data were grouped into ψ classes, and the effect of year, position, herb competition and initial
275 seedling volume was analysed by an ordinal multinomial lineal model with a generalized logit
276 link.

277 Data were checked for normality and homogeneity of variance, and were transformed,
278 when necessary, to correct deviations from these assumptions. All statistical analyses were
279 performed with the Statistica 6.0. Package (StatSoft, Inc., Tulsa, OK, USA), except the semi-
280 parametric MANOVA, which was performed with R.2.8 (R Development Core Team 2008).

281

282 **Results**

283 MICROCLIMATE, SOIL AND HERB COMMUNITY CHARACTERISTICS

284 Herbs and the *Retama* canopy reduced irradiance similarly in both years (statistical
285 results not shown). The *Retama* canopy reduced irradiance by 33%. The reduction of irradiance
286 by herbs was greater in gaps than under the *Retama* canopy (Position \times Herb competition
287 interaction, Table 1). The *Retama* canopy with herbs was the microsite with the lowest
288 irradiance. Leaf and soil temperature were significantly lower under the *Retama* canopy than in
289 gaps. Neither soil chemical properties nor relative humidity or temperature of the air
290 significantly differed among the four microsites (Table 1).

291 Soil water content in spring was 14% higher in 2007 than in 2006, while in summer it
292 was 16% lower in 2007 than in 2006 (Year \times Season interaction; $F_{1,302} = 45.63$, $P < 0.001$)
293 (Fig. 1). Soil water content was lower under the *Retama* canopy than in gaps, but this
294 difference was only observed in spring and not in summer (Position \times Season interaction; $F_{1,302}$
295 $= 5.87$, $P = 0.016$). Herbs reduced soil water content both under the *Retama* canopy and in gaps,
296 although this reduction was statistically significant in spring but not in summer (Herb
297 competition \times Season interaction; $F_{1,302} = 8.61$, $P = 0.004$). Soil water content under the
298 *Retama* canopy with herbs in spring was 20 % lower in 2006 than in 2007.

299 Herb mass was lower in 2006 than in 2007 ($F_{1,57} = 12.22$, $P = 0.01$) and under *Retama*
300 canopy than in gaps ($F_{1,57} = 11.78$, $P = 0.02$) (Table 1). However, the relative reduction of
301 herb mass under *Retama* compared to gaps was higher in 2006 than in 2007 (38% vs. 18%,

302 respectively). The composition of the herbaceous community under the *Retama* canopy
303 differed from the composition detected in gaps in 2006 but not in 2007 (Year \times Position
304 interaction; $F_{1,35} = 4.54$, $P < 0.001$). In 2006, the non-graminoid cover was significantly lower
305 under the *Retama* canopy than in gaps, whereas the graminoid cover did not differ significantly
306 between positions. By contrast, the cover of both groups of herbs were similar under the
307 *Retama* canopy and in gaps in 2007 (year \times position \times herb guild interaction; $F_{1,64} = 9.02$, $P =$
308 0.004).

309

310 *QUERCUS ILEX* SEEDLING PERFORMANCE

311 Seedling mortality of the introduced plants at the end of the experiment was 83.3% in
312 2006 and 76.9% in 2007, but these differences were not statistically significant (Table 2).
313 Significant mortality occurred in the spring of 2006, but not in the spring of 2007 (Fig. 2).
314 Seedling mortality was lower under the *Retama* canopy than in the gaps and in the absence of
315 herbs than in their presence in both years (Table 2 and Fig. 2). However, the negative effect of
316 the herbaceous community differed between years. Herbs increased seedling mortality in gaps
317 but not under *Retama* canopy in 2006, and this effect was already apparent in spring. In
318 contrast, herbs similarly increased seedling mortality at both positions in 2007, and it occurred
319 later, in summer (Year \times Herb competition \times Position interaction, Table 2). Plant size
320 negatively affected *Q. ilex* seedling mortality (Fig. 3); however, this effect was only observed
321 in gaps but not under the *Retama* canopy (Position \times Seedling size interaction in Table 2). No
322 factor influenced seedling growth (data not shown).

323 F_v/F_m was higher under the *Retama* canopy than in gaps in both years ($F_{1,76} = 5.03$, $P =$
324 0.027; Fig. 4) and higher in 2006 than in 2007 ($F_{1,76} = 5.28$, $P = 0.024$). F_v/F_m tended to be
325 higher at midday than at predawn, although this was only observed in 2007 but not in 2006
326 (Measurement time \times Year interaction, $F_{1,76}=3.79$ $P=0.052$). Neither herb competition ($F_{1,76} =$
327 0.02, $P = 0.89$) nor seedling size affected F_v/F_m ($F_{1,76} = 0.10$, $P = 0.75$).

328 Leaf water potential was higher in 2006 than in 2007 ($\chi^2=28.6$, $P < 0.001$). The
329 frequency of *Q. ilex* seedlings with higher (less negative) ψ was greater under the *Retama*
330 canopy than in gaps. This difference was more apparent in 2006 than in 2007 (Year \times Position
331 interaction, $\chi^2=6.20$, $P=0.045$; Fig. 5). Neither herb competition ($\chi^2=0.13$, $P=0.94$) nor
332 plant size ($\chi^2=5.00$, $P=0.08$) had any effect on ψ .

333

334 Discussion

335 *Retama sphaerocarpa* facilitated *Q. ilex* seedlings, which is consistent with greater
336 recruitment of *Q. ilex* under *Retama* canopy than in the gaps observed in other *Retama*
337 shrublands (Tovar 2009). This study provides further evidence for the role of facilitation in the
338 secondary succession of Mediterranean ecosystems (Maestre *et al.* 2001; Gómez-Aparicio *et*
339 *al.* 2004). However, the mechanism underlying facilitation in this system is complex since it
340 differed between years and depended on seedling size.

341

342 TEMPORAL VARIATION IN THE RELATIVE IMPORTANCE OF DIRECT AND 343 INDIRECT FACILITATION

344 In both years, *Retama* facilitated *Q. ilex* seedling survival, whereas herbs reduced it,
345 demonstrating that herbs competed with oak seedlings. However, the effects of *Retama* and
346 herbaceous vegetation on seedling survival differed between years. Herb competition started
347 earlier in the growing season in the average rainfall year than in the humid year as indicated by
348 early higher spring mortality that occurred in the former relative to the latter year. This can be
349 explained by the dryer spring of the average rainfall year in comparison to the humid year. Our
350 results suggest that there is a link between the prevailing facilitation mechanism and climate
351 conditions. Facilitation was predominantly indirect in the average rainfall year, when spring
352 was drier than in the humid year. By contrast, facilitation was direct in the humid year, when

353 summer was drier than in the average rainfall year. This assertion is supported by the following
354 results. First, herbs increased *Q. ilex* mortality in gaps but not under the *Retama* canopy in the
355 average rainfall year (Fig. 2). The lower herb mass found under *Retama* canopy as compared to
356 the gaps, specially in the average rainfall year, suggests that the nurse shrub reduced the
357 competitive capacity of the herbs, and hence indirectly improved seedling survival, as
358 previously reported in other studies (Li & Wilson 1998; Siemann & Rogers 2003; Kunstler *et*
359 *al.* 2006). Additionally, species composition of the herbaceous community differed between
360 years. In the average rainfall year, graminoids predominated over non-graminoids under the
361 nurse shrub, whereas both herb guilds had similar abundance in gaps. By contrast, in the humid
362 year, graminoid and non-graminoid cover were similar both under the *Retama* canopy and in
363 the gaps. Differences in species composition may affect the competitive capacity of the
364 community (Gordon *et al.* 1989), and graminoids tend to prevail under drought conditions
365 (Goldberg *et al.* 2001). Secondly, seedling mortality in the absence of herbs did not differ
366 between gaps and under the nurse shrub in the average rainfall year, but it did during the humid
367 year. This demonstrates lack of direct facilitation in the former case, but the existence of direct
368 facilitation in the latter case. In the average rainfall year, resource competition between the
369 nurse shrub and *Q. ilex* seedlings may have overwhelmed the direct positive effects of *Retama*
370 canopy on *Q. ilex* seedlings (Ludwig *et al.* 2004; Maestre & Cortina 2004). Third, the
371 reduction in oak seedling survival caused by herbs was similar under the shrub and in the gaps
372 in the humid year, suggesting a lack of indirect facilitation. Consistently, relative differences in
373 herb mass or in the composition of the herbaceous community were smaller or non-existent
374 between positions in the humid year respective to the average rainfall year.

375 Thus, in response to our first question, indirect facilitation may also be an important
376 mechanism for *Q. ilex* regeneration in Mediterranean shrublands that are mostly known for
377 direct facilitation (Pugnaire *et al.* 1996b; Gómez-Aparicio *et al.* 2004; Pugnaire *et al.* 2004). In
378 addition, the mechanism underlying facilitation may vary with climatic conditions. Indirect

379 facilitation can be important in years of dry springs where competition for water between nurse
380 shrubs and herbs is high, resulting in benefits for *Q. ilex* seedling establishment, whereas direct
381 facilitation mediated by microclimate amelioration seems to increase with summer aridity.
382 Therefore, not only summer drought but also spring climatic conditions may determine
383 facilitation interactions. This hypothesis should be experimentally tested in the future.

384

385 UNDERLYING MECHANISMS OF DIRECT AND INDIRECT FACILITATION

386 Variations in soil nutrient and water content do not explain the direct facilitation observed in
387 our system. Soil fertility did not differ between positions, probably because our nurse shrubs
388 were too young to have induced changes in soil properties (Pugnaire *et al.* 1996b). Soil water
389 content was lower or similar under the *Retama* canopy in comparison with the gaps, as
390 documented in other dry environments (Tielbörger & Kadmon 2000; Valladares & Pearcy
391 2002). Irradiance reduction seemed to be the main driving force for direct facilitation of *Q. ilex*
392 seedlings. The lower irradiation measured under the shrub canopy explains the lower leaf
393 temperature found in this microsite, which probably reduced *Q. ilex* transpiration under
394 *Retama* (Smith & Geller 1980). Consequently, *Q. ilex* seedlings were less water-stressed and
395 photoinhibited under the nurse shrub than in the gaps, which could explain their higher survival
396 rate under the shrubs (Hikosaka, Kato & Hirose 2004; Baquedano & Castillo 2006). The higher
397 ψ and F_v/F_m values found under *Retama* relative to gaps support this argument. Therefore, the
398 physiological results of this study provide a mechanistic explanation for the role that moderate
399 shade plays in direct facilitation in dry environments such as Mediterranean ecosystems
400 (Maestre *et al.* 2001; Gómez-Aparicio *et al.* 2005b). Seedlings were less water stressed and
401 photoinhibited (Fig. 4 and Fig. 5) in the average rainfall year than in the humid year. The
402 higher summer rainfall and soil water content in the former year can explain this difference.

403 Levine (1999) predicted that indirect facilitation is more likely when different pairs of
404 competitors compete for different resources or have different mechanisms to acquire them. The

405 three competitors of our particular system are likely to compete for the same resource, *i.e.* soil
406 water. We suggest that nurse shrubs and herbs competed mainly for soil water because the soil
407 water content under *Retama* shrubs in spring was lower than in gaps in both years. However,
408 *Retama* only suppressed herb competitive capacity in the average rainfall year, probably
409 because soil water content under the *Retama* canopy with herbs in spring was 20% lower than
410 the soil water content in the spring of the humid year. The reduction in soil water content
411 imposed by *Retama* under its canopy did not hinder *Q. ilex* performance probably because it is
412 more drought-tolerant than herbs (Levitt 1980; Larcher 1995).

413 Many studies have shown that shading by adults is the main factor responsible for
414 reducing the competition of herbs with tree seedlings (Li & Wilson 1998; Pagès *et al.* 2003;
415 Siemann & Rogers 2003; Kunstler *et al.* 2006). In our study, shade *per se* was unlikely to be a
416 major mechanism of herb suppression because shrub shade was similar in both years, but shade
417 could have exacerbated the water stress caused by low soil water content in the spring of the
418 average rainfall year (Valladares & Pearcy 2002). In contrast to our results, some previous
419 studies have found greater herb biomass under *Retama* shrubs than in gaps (Pugnaire *et al.*
420 1996b; Rodríguez-Echeverría & Pérez-Fernández 2003). This can be attributed to the
421 extremely dry climate of some regions, where facilitative interactions can overcome the
422 competitive interactions, and to the fact that these studies were performed on grazed
423 woodlands, where herbivores may reduce herb biomass to a greater extent in open spaces than
424 under shrubs (Osem, Perevolotsky & Kigel 2007).

425 Herbs and *Q. ilex* seedlings also competed for water because herbs reduced water
426 availability for the seedlings in spring (Fig. 1) as observed in previous studies (Rey Benayas *et*
427 *al.* 2005; van der Waal *et al.* 2009). This can be due to herb canopy interception of rainfall or
428 to direct exploitation by roots. Competition for water has been shown to be a major interaction
429 between herb species and tree seedlings in water-stressed systems (Knoop & Walker 1985;
430 Ludwig *et al.* 2004; van der Waal *et al.* 2009), while competition for nutrients is more

431 commonly involved in mesic systems (Pagès & Michalet 2003). Nevertheless, herbs and *Q.*
432 *ilex* seedlings might also compete for soil nutrients, and diminution of herb competition by
433 nurse shrub might have increased nutrient availability to the *Q. ilex* seedlings (Pagès *et al.*
434 2003).

435

436 RESPONSE TO FACILITATION DEPENDED ON *Q. ILEX* SEEDLING SIZE

437 The response to facilitation has been demonstrated to vary among species (Gómez-
438 Aparicio *et al.* 2004; Liancourt *et al.* 2005; Padilla & Pugnaire 2009). Our study goes a step
439 further and indicates that not all individuals within a species have the same response to
440 facilitation. Thus, amelioration of abiotic and biotic conditions by *Retama* benefited smaller
441 seedlings but not larger seedlings since *Retama* reduced mortality of smaller seedlings relative
442 to gaps but not of larger seedlings. Large seedlings frequently have lower mortality than small
443 seedlings under higher stressful conditions (Cook 1980; Leishman & Westoby 1994; Villar-
444 Salvador *et al.* 2008), such as those found in gaps. This can be explained on the basis that large
445 seedlings probably contain more nutrient and storage compounds (Cook 1980; Leishman &
446 Westoby 1994; Villar-Salvador *et al.* 2008) and have higher rates of photosynthesis (Cuesta
447 unpublished data) than smaller seedlings. Differences in seedling size within a population can
448 be attributed to genetic differences among individuals as demonstrated for *Q. ilex* (Leiva &
449 Fernández-Alés 1998), but also to emergence time or seed size (Jurado & Westoby 1992;
450 Green & Juniper 2004; De Luis, Verdú & Raventón 2008). We suggest that if *Q. ilex* seedling
451 size has a genetic basis and determines mortality probability, nurse shrubs may contribute to
452 maintain genetic diversity of *Q. ilex* populations.

453

454 CONCLUSIONS

455 In conclusion, both indirect and direct facilitation are important processes of oak
456 regeneration in *Retama* shrublands. The pioneer shrub *R. sphaerocapa* facilitated *Q. ilex*

457 seedlings both by suppressing herb competition (indirect facilitation) and/or by ameliorating
458 microclimatic conditions under its canopy (direct facilitation). Prevalence of indirect and direct
459 facilitation differed between years of distinct climatic conditions. Indirect facilitation seems to
460 be important in years of dry springs, whereas direct facilitation may increase with summer
461 aridity. Since large areas of the Iberian Peninsula have been planted with or colonized by
462 pioneer shrubs, and seedling stage is the most critical phase in Mediterranean woodland
463 regeneration, nurse shrubs could enhance shrubland conversion into forests and play an
464 important role in both passive (i.e. secondary succession) and active restoration of
465 Mediterranean woodlands (Padilla & Pugnaire 2006; Rey Benayas, Bullock & Newton 2008).
466 Furthermore, future studies should assess the incidence of indirect interactions among plants in
467 other Mediterranean shrublands, in which direct positive interactions are known to drive
468 community structure.

469

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642

643

644 **Table 1.** Microclimate features, soil properties, herb biomass and the cover of graminoid and
645 non-graminoid herbs in the four studied microsites. Microclimatic and soil variables were
646 measured in 2006. Values are mean \pm SE. Means with different superscript letters are
647 significantly different at $P < 0.05$ after a Tukey *post hoc* test. * $P < 0.05$, ** $P < 0.01$, *** $P <$
648 0.001. † Statistical data are reported in the text.
649

Variables	Microsites				Position (P)	F Herb Competition (HC)	P \times CH
	Under Retama		Gap				
	Without herbs	With Herbs	Without herbs	With herbs			
Irradiance (%)	38 \pm 1.2 ^c	28 \pm 0.9 ^d	100 \pm 0.0 ^a	46 \pm 1.5 ^b	430 ^{***}	225 ^{***}	19.9 ^{***}
Air relative humidity (%)	10.1 \pm 0.8	9.8 \pm 0.7	10.0 \pm 1.0	9.8 \pm 0.8	0.04	0.08	0.007
Air temperature (°C)	36.2 \pm 0.5	36.0 \pm 0.5	36.1 \pm 0.5	37.4 \pm 0.5	1.4	1.25	0.02
Soil temperature (°C)	30.4 \pm 1.1 ^b	28.2 \pm 0.5 ^b	41.4 \pm 0.6 ^a	39.5 \pm 1.0 ^a	179.7 ^{***}	5.82 ^{**}	0.01
Leaf temperature (°C)		43.1 \pm 2.6 ^b		49.6 \pm 1.4 ^a		t = 6.82 ^{***}	
Soil organic matter (%)		1.1 \pm 0.07		1.1 \pm 0.05		t = -0.40	
Soil N (mg kg ⁻¹)		0.8 \pm 0.05		0.8 \pm 0.1		t = -0.98	
Soil K (mg kg ⁻¹)		193 \pm 41.2		186 \pm 46.5		t = -0.32	
Soil pH		5.7 \pm 0.2		5.8 \pm 0.5		t = 0.49	
Herb mass in 2006 (g)		42 \pm 3 ^b		67 \pm 4 ^a		†	
Herb mass in 2007 (g)		67 \pm 7		81 \pm 6		†	
Graminoid cover in 2006 (%)		31 \pm 5		27 \pm 5		†	
Non-graminoid cover in 2006 (%)		4 \pm 2.0 ^b		49 \pm 7 ^a		†	
Graminoid cover in 2007 (%)		19 \pm 6		20 \pm 4		†	
Non-graminoid cover in 2007 (%)		19 \pm 4.9		26 \pm 6		†	

650 **Table 2.** Results of the ANCOVA models used to test the effects of year, position, herb
651 competition and seedling size on *Q. ilex* seedling mortality. The Year \times Position, Year \times Herb
652 competition \times Position, and Year \times Position \times Herb competition \times Seedling size interactions
653 were not analysed in spring because there was no mortality under *Retama* canopy in May
654 2007.

655

<i>Year/Factor</i>	<i>Spring</i>		<i>Summer</i>		<i>Cumulative (spring + summer)</i>	
	χ^2	<i>p</i>	χ^2	<i>p</i>	χ^2	<i>p</i>
Year (Y)	57.8	< 0.001	0.13	0.71	2.02	0.15
Herb competition (HC)	26.5	< 0.001	3.62	0.057	6.95	0.008
Seedling size (SS)	1.72	0.19	0.005	0.94	0.03	0.86
Position (P)	9.60	0.002	11.35	< 0.001	14.6	< 0.001
Y \times HC	0.51	0.47	1.35	0.24	0.309	0.58
Y \times SS	0.27	0.60	0.003	0.96	0.005	0.95
Y \times P			1.03	0.31	0.62	0.43
HC \times SS	0.34	0.58	0.0004	0.98	0.016	0.90
HC \times P	0.40	0.53	4.04	0.044	4.82	0.028
SS \times P	0.09	0.77	3.79	0.051	3.85	0.049
Y \times HC \times SS	0.59	0.44	0.87	0.35	1.16	0.28
Y \times HC \times P			3.6	0.057	3.96	0.047
Y \times SS \times P			0.56	0.45	0.52	0.47
HC \times SS \times P	0.19	0.66	0.002	0.96	0.0003	0.99
Y \times HC \times SS \times P			2.9	0.087	2.45	0.12

656

657 **Fig. 1.** Soil water content at the four microsites in 2006 (above) and 2007 (below). Data
658 represent mean values \pm SE.

659

660 **Fig. 2.** Mortality rate of *Quercus ilex* seedlings at the four microsites during spring, summer
661 and the whole first growing season in 2006 (above) and 2007 (below). Data represent means \pm
662 SE.

663

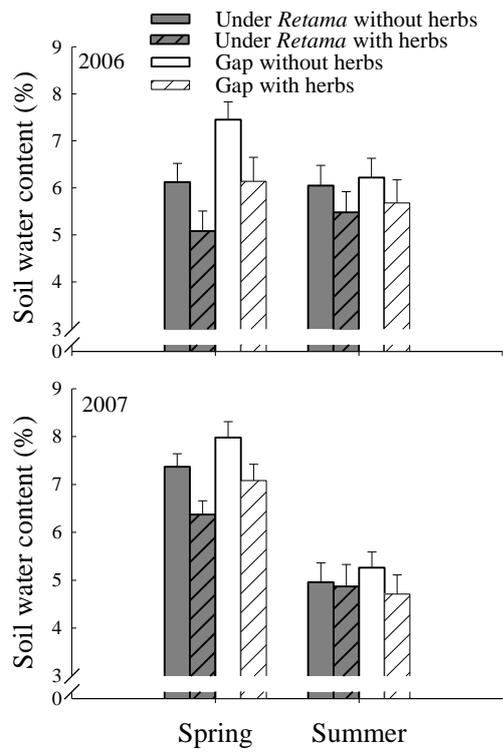
664 **Fig. 3.** Mortality probability of *Quercus ilex* seedling as a function of seedling size under the
665 *Retama* canopy and in gaps across the two years. Lines are the fitted logistic regressions. The α
666 and β parameters of the logistic regression were 2.2773 and -0.227 , respectively, for the gap
667 and 1.0087 and 0.01018, respectively, under *Retama*.

668

669 **Fig. 4.** Photochemical efficiency (Fv/Fm) of photo-system II of *Quercus ilex* seedlings planted
670 at the four microsites in 2006 and 2007. Data represent the average between morning and
671 midday data. Values are means \pm SE.

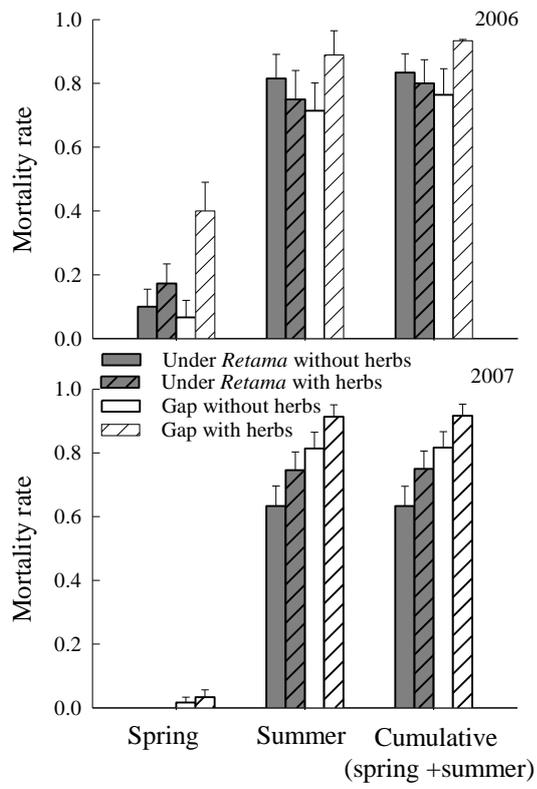
672

673 **Fig. 5.** Frequency of *Quercus ilex* seedlings according to midday leaf water potential classes
674 under the *Retama* canopy and in gaps in 2006 (top) and in 2007 (below). The number of
675 sampled plants under the *Retama* canopy and in gaps in 2006 was 12 and 10, respectively,
676 whereas 24 plants per position were sampled in 2007.



677

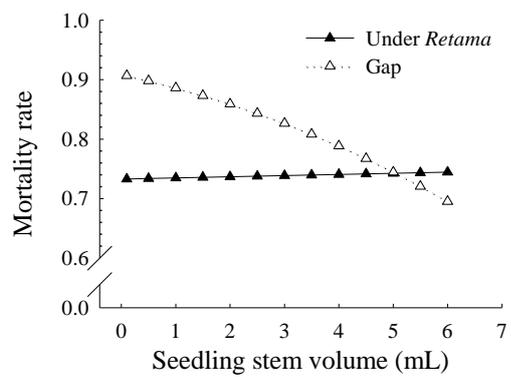
678 **Fig. 1.**



679

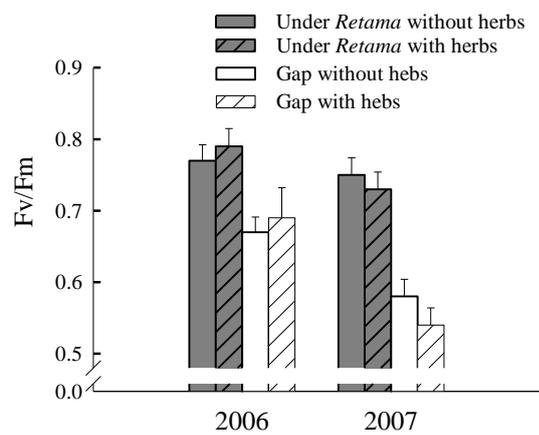
680 **Fig. 2.**

681



682

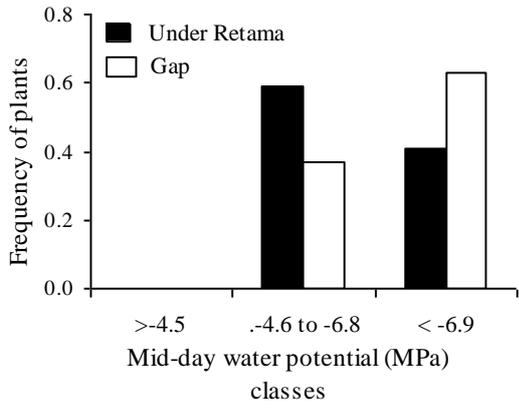
683 **Fig. 3.**



684

685 **Fig. 4.**

686



687

688 **Fig. 5**

689

690