

**Unpaved roads disrupt the effect of herbivores and pollinators on the reproduction
of a dominant shrub.**

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1 **Abstract**

2 Anthropogenic linear developments, such as trails and firebreaks, also called soft linear
3 developments (SLD), can influence animal behavior, altering the ecological interactions in
4 which animals are involved. For example, SLD can affect the behavior of pollinators and
5 herbivores, but little is known about the combined effect of these three elements on plant
6 reproduction.

7 We evaluated the combined effect of SLD, insect pollinators and herbivores (ungulates)
8 on three reproductive output variables (fruit set, seed set, and seed mass) of a Mediterranean
9 shrub (*Halimium halimifolium*). We considered two different habitats (SLD verges *vs.* adjacent
10 scrublands), two scenarios of herbivory (with and without ungulates), and three scenarios of
11 pollinator activity (without pollinators, with manual pollination and with natural pollination).

12 SLD had contrasting effects on *H. halimifolium* reproduction. In the absence of
13 herbivores, overall fruit set was lower in the verges of SLD than in adjacent scrublands,
14 probably due to lower flower pollination rates. Where herbivores were present, overall fruit set
15 was similar between habitats, because ungulate browsing was lower in SLD verges than in
16 adjacent scrublands. The quantity and weight of seeds per fruit was similar in both habitats,
17 probably because all fertilized flowers received similar amounts of pollen.

18 SLD can alter the interaction among pollinators, herbivores and plants, leading to
19 changes in the reproductive performance of the latter. These changes can have strong negative
20 impacts on endangered plants that rely on fruit and seed production to persist. However, SLD
21 verges could be safe places for plants particularly sensitive to herbivory by ungulates.

22 **Zusammenfassung**

23 Anthropogene lineare Landschaftselemente wie Wanderwege oder Feuerschneisen
24 können das Verhalten von Tieren beeinflussen und damit die ökologischen Interaktionen, an
25 denen die Arten beteiligt sind, verändern. Diese grünen linearen Strukturen (GLS) können
26 beispielsweise das Verhalten von Bestäubern und Herbivoren beeinflussen, aber wenig ist über
27 den gemeinsamen Effekt dieser drei Elemente auf die Reproduktion von Pflanzen bekannt.

28 Wir untersuchten den gemeinsamen Effekt von GLS, bestäubenden Insekten und
29 Herbivoren (freilaufende Nutz- und Wildtiere) auf drei die reproduktive Leistung
30 beschreibenden Variablen (Fruchtansatz, Samenansatz und Samengewicht) bei einem
31 mediterranen Strauch (*Halimium halimifolium*). Wir untersuchten zwei Habitattypen (Ränder
32 von GLS und benachbartes Buschland), zwei Herbivorievarianten (mit und ohne Huftiere) und
33 drei Bestäubungsszenarien (ohne Bestäuber, manuelle Bestäubung und natürliche Bestäubung).

34 GLS hatten unterschiedliche Auswirkungen auf die Reproduktion von *H. halimifolium*.

35 Bei Herbivorenausschluss war der Gesamtfruchtansatz an den Rändern der GLS geringer als im
36 Buschland, möglicherweise aufgrund von geringerer Bestäubung. Bei Anwesenheit von
37 Herbivoren war der Gesamtfruchtansatz in beiden Habitattypen ungefähr gleich, weil die
38 Huftiere an den GLS-Rändern weniger weideten als im Buschland. Die Menge und das Gewicht
39 der Samen pro Frucht waren in beiden Habitattypen ähnlich, vermutlich weil alle befruchteten
40 Blüten ähnliche Mengen von Pollen erhielten.

41 GLS können die Interaktionen zwischen Bestäubern, Herbivoren und Pflanzen
42 verändern, was zu veränderter reproduktiver Leistung bei den Pflanzen führt. Diese
43 Veränderungen können stark negative Auswirkungen auf gefährdete Pflanzenarten haben, die
44 auf Frucht- und Samenproduktion angewiesen sind, um zu persistieren. Indessen können GLS-
45 Ränder sichere Orte für Pflanzen sein, die gegen Pflanzenfraß durch Huftiere empfindlich sind.

46 **Keywords:** ecosystem services, fruit set, *Halimium halimifolium*, herbivory,
47 pollination, trails.

48 **Introduction**

49 Human-induced changes in plant and/or pollinator populations often lead to the
50 disruption of pollination mutualisms and consequently to pollen limitation (Ashman,
51 Knight, Steets, Amarasekare, Burd et al. 2004; Gómez, Abdelaziz, Lorite, Muñoz-
52 Pajares & Perfectti 2010; González-Varo, Arroyo & Aparicio 2009). Pollen limitation
53 leads to decreased fruit and seed production in countless crops and wild plants, which
54 negatively affects agricultural yields (Garibaldi, Aizen, Klein, Cunningham & Harder
55 2011) as well as the colonization ability and the persistence of wild plants (Biesmeijer,
56 Roberts, Reemer, Ohlemüller, Edwards et al. 2006; Bond 1994; Burd 1994).

57 Understanding how and to what extent human activities affect plant-pollinator
58 interactions and, ultimately, plant reproductive performance, is therefore a priority for
59 conserving the supply of goods and services that plants provide (Biesmeijer et al. 2006;
60 Bond 1994; Isbell, Calcagno, Hector, Connolly, Harpole et al. 2011).

61 At a global scale, human activities have led to the so-called “pollination crisis”
62 (Kearns, Inouye & Waser 1998). More specifically, pollinators are declining due to
63 habitat loss and fragmentation (i.e. reduced availability of feeding and nesting
64 resources), agriculture intensification (which usually involves pesticide and herbicide
65 application), and climate change, in addition to the spread of pests, pathogens and alien
66 species (Potts, Biesmeijer, Kremen, Neumann, Schweiger et al. 2010). At smaller
67 scales, pollinator abundance and behavior can be negatively influenced by local
68 anthropogenic habitat transformation (Elliott, Lindenmayer, Cunningham & Young
69 2012; Kennedy, Lonsdorf, Neel, Williams, Ricketts et al. 2013; Winfree, Aguilar,
70 Vázquez, LeBuhn & Aizen 2009).

71 One of the most pervasive human-mediated habitat transformations is the
72 construction of paved roads (e.g. highways), as well as similar structures that involve

73 minor habitat transformation such as trails and firebreaks (also known as “Soft Linear
74 Developments”, SLD; Suárez-Esteban, Delibes & Fedriani 2013a). Paved roads can
75 have contrasting effects on plant pollination. For example, they can reduce the
76 frequency of pollinating birds visiting flowers (Geerts & Pauw 2011; but see Francis,
77 Kleist, Ortega & Cruz 2012; Magrach, Guitián & Larrinaga 2011). On the other hand,
78 roadsides often host flourishing grasslands that provide suitable habitats for diverse and
79 abundant populations of pollinators (Hopwood 2008; Noordijk, Delille, Schaffers &
80 Sýkora 2009).

81 SLD are as pervasive as paved roads (e.g. Pasher, Seed & Duffe 2013).
82 However, very few studies have assessed their effects on plant reproduction. For
83 example, SLD can result in increased habitat fragmentation and edge effects, such as
84 dust deposition on flowers, extreme climate conditions and pollinator mortality
85 (Cunningham 2000; Huang, Sun, Yu, Luo, Hutchings et al. 2009; Jules & Rathcke
86 1999; Kolb 2008; but see Magrach, Santamaría & Larrinaga 2013), that can reduce the
87 abundance of plants and pollinators, and also affect the behavior of the latter. On the
88 other hand, shrub hedgerows may exist in the verges of SLD, either because they were
89 planted (Karim & Mallik 2008) or because they established naturally (Suárez-Esteban,
90 Delibes & Fedriani 2013b). In such cases, SLD might assist pollination, as hedgerows
91 are known to provide food resources and shelter for pollinators (Morandin & Kremen
92 2013; Schmucki & De Blois 2009). Interestingly, SLD can also modify the behavior of
93 large herbivores such as ungulates. Wild ungulates usually avoid SLD and other similar
94 habitat edges (Cadenasso & Pickett 2000; Suárez-Esteban et al. 2013a). Therefore, SLD
95 could buffer herbivore damage on plant reproduction (e.g. predation of leaves, flowers,
96 unripe fruits or whole plants), limiting the loss of plant attractiveness for pollinators
97 (Gómez 2003).

98 Although both pollinators and ungulates can be affected by SLD (Huang et al.
99 2009; James & Stuart-Smith 2000), and all these three elements pervasively co-occur in
100 the wild, no prior study has assessed their combined effect on plant reproduction. A
101 better understanding on the potential of SLD to disrupt key plant-animal interactions
102 (e.g. pollination, herbivory) will allow us to better predict the effects of SLD and
103 increase our efficiency when planning SLD networks. We specifically chose to evaluate
104 the combined effect of SLD, ungulate herbivores (e.g. deer, cattle) and pollinators on
105 the reproductive output of the common shrub *Halimium halimifolium* L. (Cistaceae) in
106 several patches of Mediterranean scrubland. We compare three measures of
107 reproductive output (fruit set, seed set, and seed mass) in naturally-pollinated flowers
108 between two habitats (SLD verges and scrubland) under two scenarios of herbivory
109 (with and without ungulates). In order to link reproductive output and pollination
110 (Cunningham 2000), we compared flowers exposed to different pollination scenarios
111 (without pollinators, with manual pollination and with natural pollination).

112 With this experimental design, we aimed to answer the following questions: does
113 herbivory by ungulates limit the reproductive output of *H. halimifolium*? Are the effects
114 of ungulates conditioned by the presence of SLD? Is the reproductive output of *H.*
115 *halimifolium* dependent upon pollination? Is this species' reproductive output affected
116 by SLD? Based on the literature, the net effect of SLD on *H. halimifolium* predispersal
117 reproductive performance is difficult to anticipate.

118

119 **Materials and methods**

120 **Study system**

121 This study was carried out during April-August of 2010 and 2011 in Doñana
122 National Park (SW Spain; 37° 9' N, 6° 26' W; 510 km²; elevation 0-80 m). The climate

123 is Mediterranean sub-humid, characterized by dry, warm summers (June–September)
124 and mild, wet winters (December–March). Annual rainfall is irregular, averaging 577
125 mm \pm 39 SE, with 88.4% of rain falling between October and April (data from Natural
126 Processes Monitoring Group, Doñana Biological Station, [http://www-](http://www-rbd.ebd.csic.es/Seguimiento/seguimiento.htm)
127 rbd.ebd.csic.es/Seguimiento/seguimiento.htm).

128 The Doñana area contains several habitats (e.g. marshland, scrubland, dunes)
129 and a vast SLD system (over 2,000 km) composed mainly of dirt trails (62.5%) and
130 firebreaks (35.5%). The scrubland patches harbor a diverse community of native
131 Mediterranean shrubs. This community of shrubs includes our model species, *Halimium*
132 *halimifolium* L., an abundant Cistaceae shrub that grows in a wide range of
133 environmental conditions in the Western Mediterranean. At Doñana, it dominates
134 extensive scrubland patches from the edges of marshlands to mobile dunes (Díaz
135 Barradas, Zunzunegui & García Novo 1999). Throughout the study area, it reaches
136 similarly high densities in both SLD verges (10.14 ± 1.33 mean number of
137 individuals/m² \pm SE) and in adjacent scrublands (9.79 ± 1.13 ; Suárez-Esteban et al.
138 2013b).

139 *H. halimifolium* has large (up to 62 mm in diameter) hermaphrodite yellow
140 flowers that bloom mainly in late-spring and early-summer (May-June; Herrera 1988).
141 Flowers emerge as apical inflorescences and are pollinated mainly by pollen-eating
142 beetles, such as *Heliotaurus ruficollis* Fabricius (Tenebrionidae), and less frequently by
143 some bees (Halictidae; (Herrera 1986). Both beetles and bees are usually observed
144 feeding on *H. halimifolium* flowers both near and far from SLD in our study area
145 (*Authors personal observation*). Fertilized flowers lead to small dry fruits, containing 25
146 seeds on average (Herrera 1987a). After the fruiting period, fruits dry up and usually
147 drop their seeds on the ground. Although no evident specialized dispersal mechanism is

148 observed, this species seems to have a great colonization ability, as documented in other
149 Cistaceae species (Bastida & Talavera 2002).

150 Within our study area, browsing ungulates such as red deer (*Cervus elaphus* L.),
151 fallow deer (*Dama dama* L.), and livestock (e.g. cattle, horses) reach high densities,
152 because of the absence of both hunting pressure and natural predators. These ungulates
153 can severely damage *H. halimifolium* (Silva, Barradas & Zunzunegui 1996).

154 **Experimental design**

155 Because reproductive output can vary in time and space, we surveyed three
156 different study sites separated from each other by 2.5 – 14 km that are called “Reserva”,
157 “Rocina” and “Matasgordas” (described in detail in Suárez-Esteban et al. 2013a), during
158 two consecutive years (2010 and 2011). At each site, we set up two independent
159 experimental blocks separated by a minimum of 1 km (Fig. 1). Within each
160 experimental block we set up four plots (~ 36 m² each). Two of the plots were located
161 along SLD verges and the other two were located in the scrubland, 60 meters away from
162 SLD (since most edge effects cease to be significant within 50 m; Murcia 1995; Fig. 1).
163 Of the two plots established in each habitat, one was fenced to exclude large herbivores
164 (i.e. ungulates such as deer and cattle), while another was left completely open (Fig. 1).
165 Open and fenced plots within each habitat were adjacent, ensuring that they were
166 subjected to the same environmental conditions. In total, we established 24 plots (12 in
167 SLD verges, 12 in the scrubland), all of them containing at least ten reproductive *H.*
168 *halimifolium* individuals.

169 To evaluate whether herbivory by ungulates affected the reproductive output of
170 *H. halimifolium* both near and far from SLD verges, we compared fruit set, seed set, and
171 seed mass between open and fenced plots in SLD verges and in the scrubland. To assess
172 whether SLD alone affected the reproductive output of *H. halimifolium*, we also

173 compared the same three reproductive measures between fenced plots (i.e. controlling
174 for the effect of herbivory) in SLD verges and in the scrubland.

175 Linking habitat differences in reproductive output with differential pollination
176 requires confirmation that pollen reception limits the reproductive output (Cunningham
177 2000). To do this, in each plot we simulated three scenarios of pollinator activity:

178 a) Without pollinators:

179 After counting the flower-buds, we tagged and bagged a number of
180 inflorescences (five in 2010 and two in 2011) of each of five randomly selected *H.*
181 *halimifolium* plants within each plot to preclude pollinator access (Parker 1997).
182 Overall, we tagged and bagged 779 inflorescences with 53152 flowers. After the
183 flowering period (July-August), we counted the resulting fruits and randomly sampled
184 three of them from each bagged inflorescence. All collected fruits were stored in paper
185 bags, then dissected in the lab to count the number of seeds produced per fruit and to
186 measure their mass (using a precision scale).

187 b) Manually supplemented cross-pollination:

188 Using the same five *H. halimifolium* plants mentioned above, we randomly
189 selected five open inflorescences that were different from those that had been bagged to
190 exclude pollinators. For each of these open inflorescences, we added supplemental
191 pollen to one randomly selected flower per inflorescence (hereafter “supplemented
192 flower”), by rubbing its stigma with collected anthers from distant (20-30 m)
193 conspecifics until it was completely covered with pollen.

194 Pollen addition may overestimate the magnitude of pollen limitation if plants
195 reallocate resources from non-manipulated flowers to supplemented flowers (Haig &
196 Westoby 1988; Knight, Steets & Ashman 2006). To assess whether such resource
197 reallocation occurs (thereby potentially affecting our results), for each supplemented

198 flower we labeled three control flowers: one within the same inflorescence as the
199 supplemented flower (hereafter “intra-inflorescence control”), one within another
200 inflorescence on the same plant (hereafter “inter-inflorescence control”), and the last
201 one on another adjacent individual, outside the plot (hereafter “external control”). All
202 control flowers were exposed to natural pollination (no pollen was added). For this, we
203 tagged and monitored 1034 flowers for each of the four treatments (i.e. supplemented
204 cross-pollination and three controls). After the flowering period, we counted all
205 resulting fruits set by supplemented and control flowers, harvesting and processing all
206 of them as above.

207 c) Natural pollination

208 To estimate *H. halimifolium* natural fruit set, we tagged five inflorescences
209 (when possible) of each of five additional *H. halimifolium* plants (different from the five
210 plants used to simulate the absence of pollinators and the effect of supplemented cross-
211 pollination) within each plot and counted their flowers, which were exposed to natural
212 pollination (hereafter “naturally-pollinated flowers”). Overall, we tagged 1149
213 inflorescences with 80038 flowers. As above, we counted the number of fruits set by all
214 tagged inflorescences and randomly sampled three fruits from each. In the 2011
215 surveys, we selected the same individuals used in 2010 when possible.

216 **Statistical analyses**

217 Fruit set of bagged flowers (i.e. those that were not exposed to pollinators) was
218 ~2% in both habitats, confirming that *H. halimifolium* reproductive output relies on
219 animal pollination. Because of its small sample size, we excluded this treatment from
220 the analyses.

221 Using data on the supplemented flowers, we evaluated whether habitat,
222 herbivory or their interaction altered the importance of pollination for *H. halimifolium*

223 reproductive output. To address the effect of habitat, we compared plots in SLD verges
224 *vs.* plots in the scrubland. To address the effect of herbivory by ungulates, we compared
225 open *vs.* fenced plots. Finally, to quantify a potential interaction between habitat and
226 herbivory, we compared SLD verges with the scrubland in terms of any differences
227 between open and fenced plots.

228 We fitted three generalized linear mixed models (GLMMs) with three different
229 response variables: (1) the proportion of flowers that set fruits, i.e. fruit set, per plant
230 (using a binomial error-distribution and logit-link function), (2) the number of seeds
231 produced per fruit, i.e. seed set (using a negative binomial distribution and log-link
232 function), and (3) the overall seed mass per fruit (using a Gaussian distribution and
233 identity-link function). In these models, we also included data on control flowers to
234 assess the potential occurrence of resource reallocation.

235 Using data on natural pollination, we evaluated the effect of habitat, herbivory
236 and their interaction on the reproductive output of naturally-pollinated flowers. To do
237 so, we fitted three additional GLMMs with the same response variables as above (i.e.
238 fruit set per plant, seed set per fruit and seed mass per fruit).

239 All GLMMs were implemented using the SAS 9.2 GLIMMIX procedure (Littell,
240 Milliken, Stroup, Wolfinger & Schabenberger 2006) and adjusted using maximum
241 likelihood (Bolker, Brooks, Clark, Geange, Poulsen et al. 2009). In all models we
242 considered the habitat (SLD verges *vs.* scrubland), herbivory by ungulates (open *vs.*
243 fenced plots), and their interaction as fixed factors. When analyzing whether pollen
244 reception and resource reallocation varied among factor combinations, we also included
245 the pollination treatment (i.e. supplemented *vs.* control flowers; referred to as
246 “Supplement” in Table 1) and its interactions with habitat and herbivory as fixed
247 factors. When any interaction was significant, we performed tests for the effect of a

248 factor at the different levels of the other factor (“tests of simple main effects”) using the
249 SLICE option in the LSMEANS statement (Littell et al. 2006).

250 The use of GLMMs allowed us to model non-normal variables as well as to
251 introduce random factors in order to account for temporal and spatial heterogeneity, in
252 addition to potential individual effects that might influence our results. Specifically, we
253 included the year, the study site, the plot (nested within site), and the plant (nested
254 within plot) as random factors. For every model concerning seed set and seed mass, we
255 removed from the dataset all fruits that presented some fault (e.g. with depredated,
256 unripe or rotten seeds), which happened rarely.

257 **Results**

258 **Supplemented cross-pollination**

259 Pollen supplementation increased fruit set by 114.6%, seed set by 35.0% and
260 seed mass by 29.0%, as compared with control (naturally-pollinated) flowers (see
261 “Supplement” in Table 1; Fig. 3). This suggests that the reproductive output of *H.*
262 *halimifolium* (all three variables) is generally limited under natural conditions. As found
263 for naturally-pollinated flowers, fruit set of both supplemented and control flowers was
264 slightly higher in fenced plots than in open plots in the scrubland, although these
265 differences were only marginally significant (Table 1).

266 With respect to the possibility of resource reallocation, control flowers showed
267 similar ($P > 0.05$) fruit set (Fig. 2), seed set (Fig. 3) and seed mass. This indicates the
268 lack of resource reallocation in *H. halimifolium* at any scale, reinforcing the strength of
269 our inference on the link between pollen reception and reproductive output in our study
270 system.

271 The effect of pollen supplementation on fruit set was consistent in both habitats,
272 and in both fenced and open plots (i.e. habitat, herbivory and their interactions with the

273 pollination treatment were not statistically different between either habitats or plots;
274 Table 1). However, when analyzing seed set and seed mass, we found marginally
275 significant differences between habitats (see “Habitat” in Table 1). These marginal
276 differences were the result of a higher seed set and seed mass of pollen-supplemented
277 flowers in the scrubland than in SLD verges (see Fig. 3). We did not find any other
278 significant effect of habitat or herbivory on seed set or seed mass (Table 1).

279 **Natural pollination**

280 Naturally-pollinated flowers set 27689 fruits (overall fruit set 34.6%; n =
281 80038). Habitat and herbivory did not significantly affect fruit set (Table 1). However,
282 the interaction between habitat and herbivory was significant (Table 1), suggesting that
283 the effect of ungulates differed between habitats. Indeed, the exclusion of ungulates had
284 a significant effect in the scrubland, but not in SLD verges. In SLD verges we found no
285 differences in the fruit set between open and fenced plots. In the scrubland, however,
286 fruit set was 33.0% higher in fenced than in open plots (Fig. 4). Considering exclusively
287 the differences between fenced plots in both habitats (i.e. controlling for the effect of
288 herbivory), the fruit set per plant was a 33.1% higher in the scrubland than in SLD
289 verges (test of slices: $F_{1,113} = 6.90$; $P < 0.01$; Fig. 4). Thus, SLD negatively affected *H.*
290 *halimifolium* fruit set in absence of ungulates.

291 We collected 2612 fruits (9.43% of the counted fruits) to estimate the number of
292 seeds and their overall mass per fruit of naturally-pollinated flowers. The number of
293 seeds produced per fruit ranged between 1 and 72. The mean number of seeds produced
294 per fruit was similar between SLD verges (20.12 ± 1.81 ; mean \pm SE) and adjacent
295 scrublands (21.87 ± 1.96 ; Table 1). We did not find a significant effect of herbivory by
296 ungulates on seed set (Table 1). This lack of ungulate effect was consistent in both
297 habitats (the interaction Habitat*Herbivory was not significant; Table 1).

298 The overall seed mass per fruit ranged between 0.1 and 31.4 mg. As was true for
299 the average seed set, seed mass was similar between SLD verges (8.97 ± 0.59 mg; mean
300 \pm SE) and adjacent scrublands (9.80 ± 0.59 mg). We did not detect any significant effect
301 of habitat, herbivory, or their interaction on seed mass (Table 1). These results for both
302 seed set and seed mass suggest that SLD did not affect seed production.

303 **Discussion**

304 **Effects of SLD on pollination**

305 As found by Herrera (1987a), bagged *H. halimifolium* barely produced fruits in
306 either habitat. Furthermore, Herrera (1987b) found that self-pollinated flowers of a
307 closely related species (*H. calycinum*) did not set any fruit. This evidence suggests that
308 *H. halimifolium* is highly dependent on cross-pollination mediated by insects regardless
309 of the presence of SLD. *H. halimifolium* did not reallocate resources in response to
310 pollen supplementation. This confirms that the more pollen a plant receives, the more
311 fruits and seeds it produces, i.e. *H. halimifolium* is pollen-limited, as found for other
312 species (Burd 1994; Parker 1997). This pollen-reception dependence was consistent in
313 both habitats.

314 We found that, in the absence of herbivores, plants in SLD verges produced
315 relatively fewer fruits despite having a similar seed yield (i.e. seed number and mass)
316 per fruit than plants in the scrubland. Given that pollen-reception is positively correlated
317 with both fruit and seed production, our results suggest that the proportion of pollinated
318 flowers was lower in SLD verges than in the scrubland (which resulted in a lower fruit
319 set), but all pollinated flowers received similar amounts of pollen, regardless of the
320 habitat (which explains the consistency of seed set and seed mass between habitats).

321 Lower flower pollination rates in SLD verges relative to adjacent scrubland in
322 the absence of herbivores can be related to the reception of fewer or poorer quality

323 pollen grains (Aizen & Harder 2007). Lower pollen arrival is usually associated with
324 either lower visitation rates by pollinators (Kolb 2008; Parker 1997) or with lower
325 pollinator efficiency when removing and transporting pollen, e.g. due to briefer visits
326 (Aizen et al. 2007; Wilson & Thomson 1991). As our results suggest that all pollinated
327 flowers received a similar amount of pollen, we assume that pollinator efficiency did
328 not vary between habitats. Thus, we propose that this pattern is largely explained by
329 lower visitation rates.

330 Flower visitation rates by pollinators can be affected by abiotic conditions (Jules
331 et al. 1999). As a matter of fact, we observed that *H. halimifolium* flowers tended to
332 close with windy weather. Wind turbulence and exposure is probably much higher at
333 SLD verges, given the lack of surrounding, protective vegetation. This probably
334 decreased the period of time during which *H. halimifolium* flowers along SLD were
335 available relative to those within the scrubland. Reduced availability of open flowers
336 could have reduced the likelihood of pollen reception and fecundation in SLD due to
337 both a reduced time of exposure for each flower, as well as an overall reduction in
338 attractiveness for pollinators. Furthermore, wind intensity and turbulence can hinder
339 pollinator activity (Sayre, Kelty, Simmons, Clayton, Kassam et al. 2013), which could
340 act in synergy with the reduced availability of flowers.

341 Considering that in the absence of herbivores, naturally-pollinated flowers set
342 more fruits in the scrubland than along SLD verges, the overall production of seeds in
343 the scrubland was higher than in SLD verges. Although the number of seeds per fruit set
344 by naturally-pollinated flowers was similar in both habitats, we found that pollen-
345 supplemented flowers set a significantly higher number of seeds per fruit in the
346 scrubland than along SLD verges (see “Suppl” in Fig. 3). This suggests the existence of

347 other effects of SLD (e.g. dust deposition and interference with pollen, Lewis, Schupp
348 & Monaco 2012) that would affect negatively the seed set when pollen is unlimited.

349 **Combined effect of SLD and ungulates on plant reproduction**

350 Large ungulates such as red deer and caribou (*Rangifer tarandus*) usually avoid
351 linear structures such as SLD (James et al. 2000; Suárez-Esteban et al. 2013a).
352 Accordingly, we only found a negative effect of ungulates on *H. halimifolium* fruit set
353 in the scrubland (Fig. 4). Thus, SLD had a partly beneficial effect on *H. halimifolium*
354 reproductive output. We did not detect any significant effect of herbivory by ungulates
355 on the number of seeds produced per fruit. This suggests that ungulates mainly disrupt
356 fruit set, probably by removing flowers and entire inflorescences (Vázquez &
357 Simberloff 2004). By reducing the fruit set in the scrubland, ungulates diminished in
358 turn the overall production of seeds in that habitat, but not at SLD verges. However, this
359 potentially positive effect of SLD was counterbalanced by other negative effects on
360 pollination (see above). We expect this reduction in herbivory mediated by SLD will be
361 rather advantageous in other plant ontogenetic stages, such as the seedling and the
362 sapling stage (Cadenasso et al. 2000).

363 **Conclusions and implications**

364 Effective management of plant populations in anthropic ecosystems requires an
365 understanding of the ecological drivers of plant reproduction and how they interact with
366 pervasive human features. Our results suggest that SLD can have negative effects on
367 local pollination and thereby on the reproductive output of *H. halimifolium*, a dominant
368 Mediterranean shrub. However, limited fruit or seed production do not necessarily mean
369 a negative effect on plant populations (Herrera, Medrano, Rey, Sánchez-Lafuente,
370 García et al. 2002). Negative effects of SLD on pollination might be overcome by
371 positive effects at other stages such as seed dispersal (Suárez-Esteban et al. 2013a),

372 while the resulting net effect will ultimately determine plant fitness (Magrach et al.
373 2013). Despite the importance of considering all ontogenetic stages as a whole, very
374 little is known about whether SLD affect seed predation, germination, and seedling
375 establishment (Ogden, Heynen, Oslander, West, Kassam et al. 2013; but see Suárez-
376 Esteban 2013). Thus, further research is clearly needed.

377 Given that *H. halimifolium* readily colonizes SLD after one year without
378 perturbations (*Authors personal observation*), fruit and seed set do not seem to be the
379 most limiting factors for population size. Rather, the availability of empty sites without
380 competitors is more likely to influence *H. halimifolium* dynamics. However, the fact
381 that we detected a negative effect of SLD on the reproductive output of such locally
382 widespread and abundant species suggests that SLD disturbances could have a deeper
383 impact on other taxa (Cunningham 2000). For instance, those plants with small
384 populations, depending upon specialist pollinators sensitive to SLD derived effects (e.g.
385 wind, dust), and those whose fitness relies mainly on sexual reproduction, will likely be
386 significantly deterred by SLD. On the other hand, SLD could have a positive effect on
387 plant populations whose viability is negatively affected by ungulates. Therefore, careful
388 SLD development in conjunction with further research is desirable to effectively
389 manage sensitive plant populations.

390 SLD effects are likely heterogeneous among different species (i.e. SLD effects
391 are context-dependent; Suárez-Esteban et al. 2013b). This highlights the importance of
392 local scale studies that can be translated into management practices. In order to reduce
393 the negative effects of SLD on pollination, we propose the conservation of roadside
394 native vegetation. SLD verges have the potential to host shrubby hedgerows (Suárez-
395 Esteban et al. 2013b) that can act as wind and dust screens, as well as provide
396 pollinators with habitat for both foraging or nesting (Morandin et al. 2013; Mwangi,

397 Kasina, Nderitu, Hagen, Gikungu et al. 2012). Natural hedgerows along roadsides can
398 be achieved by conserving endozoochorous seed dispersal vectors that positively select
399 SLD verges for defecation (Suárez-Esteban et al. 2013a) and by promoting shrub
400 establishment along SLD verges (Karim et al. 2008; Suárez-Esteban et al. 2013b).

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566

567 **TABLES**

568 **Table 1.** Results of the GLMMs fitted for testing the effects of habitat, herbivory, pollen-supplementation (Supplement) and their interactions on
 569 the fruit set, seed set and seed mass. *P*-values are represented by asterisks (m.s. $P < 0.07$; * $P < 0.05$; *** $P < 0.0001$).

| Factor | Supplemented cross-pollination | | | | | | Natural pollination | | | | | |
|----------------|--------------------------------|----------------------|----------|----------------------|-----------|----------------------|---------------------|-------|----------|-------|-----------|------|
| | Fruit set | | Seed set | | Seed mass | | Fruit set | | Seed set | | Seed mass | |
| | df | F | df | F | df | F | df | F | df | F | df | F |
| Habitat | 1,763 | 0.16 | 1,1369 | 3.73 ^{m.s.} | 1,1369 | 3.73 ^{m.s.} | 1,113 | 2.72 | 1,2491 | 1.90 | 1,2491 | 1.61 |
| Herbivory | 1,763 | 0.23 | 1,1369 | 2.27 | 1,1369 | 2.04 | 1,113 | 2.71 | 1,2491 | 0.002 | 1,2491 | 0.08 |
| Supplement | 3,763 | 127.89*** | 3,1369 | 39.04*** | 3,1369 | 34.86*** | - | - | - | - | - | - |
| Hab*Suppl | 3,763 | 2.19 | 3,1369 | 0.63 | 3,1369 | 1.57 | - | - | - | - | - | - |
| Herb* Suppl | 3,763 | 1.74 | 3,1369 | 1.62 | 3,1369 | 1.01 | - | - | - | - | - | - |
| Hab*Herb | 1,763 | 3.29 ^{m.s.} | 1,1369 | 0.00 | 1,1369 | 0.34 | 1,113 | 4.34* | 1,2491 | 0.13 | 1,2491 | 0.04 |
| Hab*Herb*Suppl | 3,763 | 0.45 | 3,1369 | 1.35 | 3,1369 | 0.88 | - | - | - | - | - | - |

570

Figure captions

Fig. 1. Spatial distribution of our three study sites inside Doñana National Park (SW Spain). In each of the three sites, we set up two experimental blocks, with open and fenced plots both along SLD (dark grey stripe) verges and in the scrubland, as shown by the diagram in the top-right.

Fig. 2. Model-adjusted means (\pm SE) of fruit set by pollen-supplemented (“Suppl”) and control flowers (from left to right: intra-inflorescence, inter-inflorescence and external controls) along SLD verges (black bars) and in the scrubland (white bars). We only found significant differences between supplemented (a) and all types of control flowers (b). No significant differences were found between habitats (n.s. = non significant, $P > 0.05$).

Fig. 3. Model-adjusted means (\pm SE) of seed set by pollen-supplemented (“Suppl”) and control flowers (from left to right: intra-inflorescence, inter-inflorescence and external controls) along SLD verges (black bars) and in the scrubland (white bars). We only found significant differences between supplemented and control flowers. No significant variations were found between habitats, though the overall seed set was slightly higher in the scrubland as compared with SLD verges (** $P < 0.01$; m.s. $P = 0.054$; n.s. = non significant, $P > 0.054$).

Fig. 4. Model-adjusted means (\pm SE) of fruit set by naturally-pollinated flowers along SLD verges (black bars) and in the scrubland (white bars) between open and fenced plots (i.e. with and without ungulates, respectively). Letters show whether differences between least-square means were significant (n.s. = non significant).

FIGURE 1

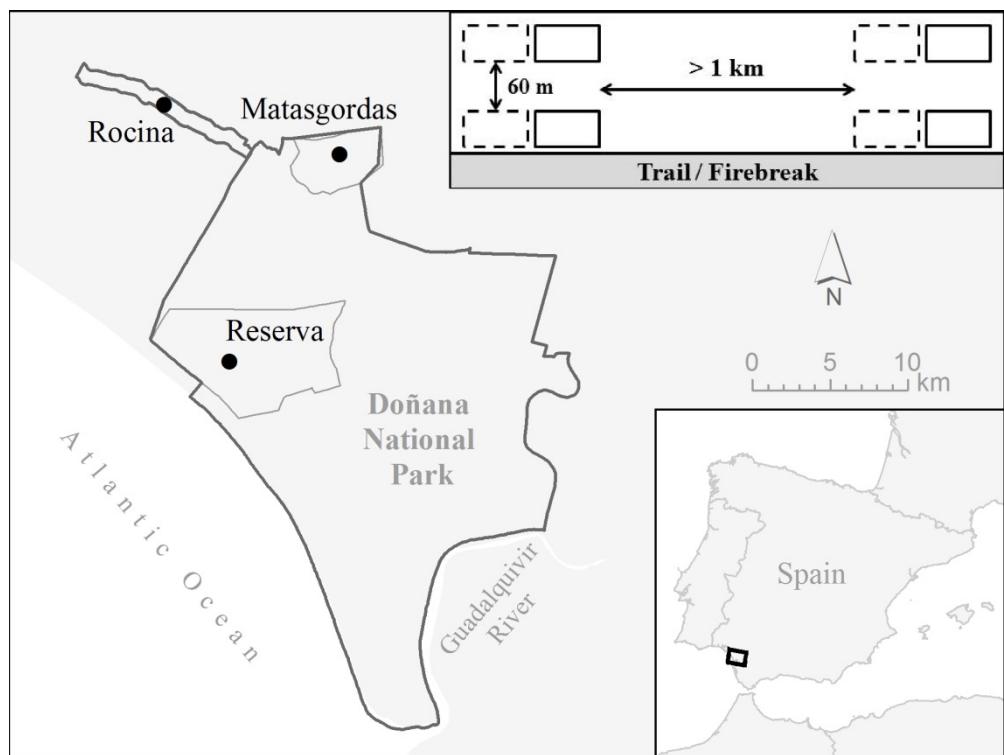


FIGURE 2

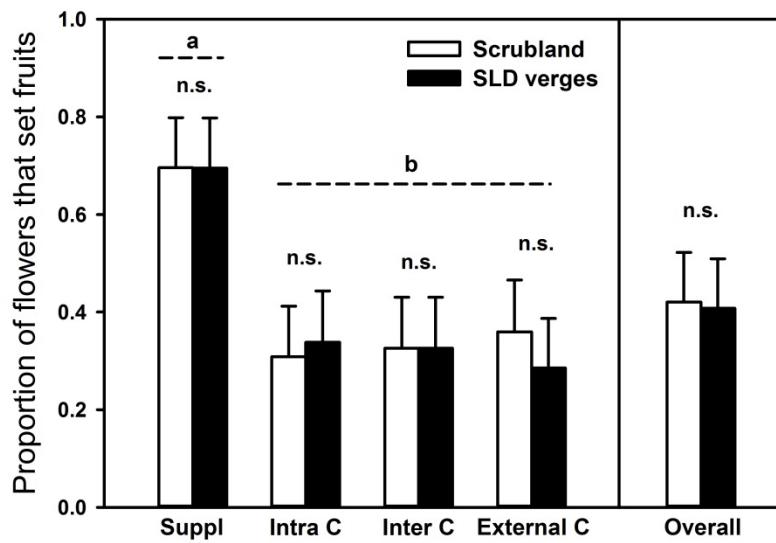


FIGURE 3

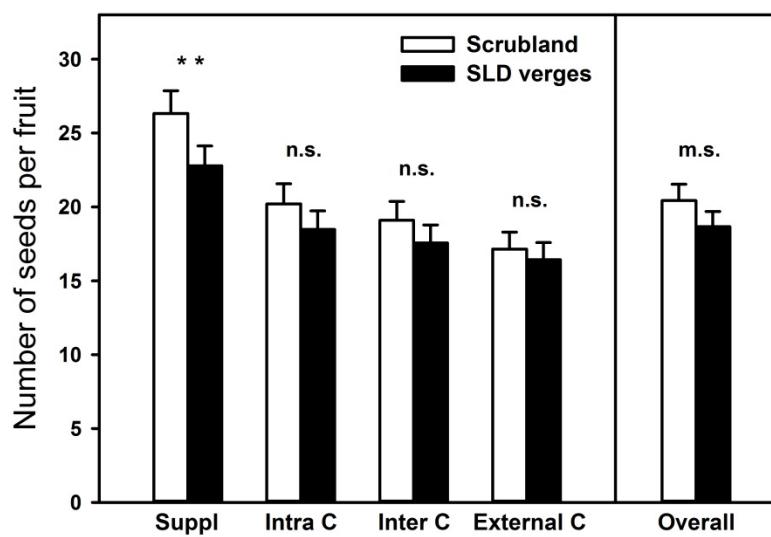


FIGURE 4

