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1 **Ants indirectly reduce the reproductive performance of a leafless shrub by benefiting**  
2 **aphids through predator deterrence**

3

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26 **Abstract**

27 Ant-aphid mutualisms can generate cascade effects on the host plants, but these impacts  
28 depend on the ecological context. We studied the consequences of ant-aphid interactions on  
29 the reproductive performance of a Mediterranean leafless shrub (*Retama sphaerocarpa*),  
30 through direct and indirect effects on the arthropod community. By manipulating the presence  
31 of ants and aphids in the field, we found that ants increased aphid abundance and their  
32 persistence on the plant and reduced aphid predators by nearly half. However, the presence of  
33 ants did not affect the abundance of other plant herbivores, which were relatively scarce in the  
34 studied plants. Aphids, and particularly those tended by ants, had a negative impact on the  
35 plant reproductive performance by significantly reducing the number of fruits produced.  
36 However, fruit and seed traits were not changed by the presence of aphids or those tended by  
37 ants. We show that ants favoured aphids by protecting them from their natural enemies but  
38 did not indirectly benefit plants through herbivory suppression, resulting in a net negative  
39 impact on the plant reproductive performance. Our study suggests that the benefits obtained  
40 by plants from hosting ant-aphid mutualisms are dependent on the arthropod community and  
41 plant traits.

42

43 **Key words.** Ant-aphid mutualism, herbivory, indirect interactions, *Retama sphaerocarpa*,  
44 seed production, top-down effects.

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51 **Introduction**

52 Trophic interactions are key determinants of ecosystem functioning, population dynamics and  
53 community structure (Ings et al. 2009). Many outcomes of these interactions are difficult to  
54 anticipate as they include mutualistic and antagonistic species interactions that cannot be  
55 studied by pairwise interactions alone (Reiss et al. 2009). More complex approaches are  
56 needed to integrate positive and negative links as well as direct and indirect effects among  
57 several trophic levels (Clark et al. 2016; Seibold et al. 2018). Individual plants can host a  
58 wide range of arthropods that are engaged in multiple interaction types, and each interaction  
59 may have different impacts on host-plant performance (Ando et al. 2017). A keystone  
60 interaction affecting several ecological processes in plants is the mutualism between ants and  
61 aphids (Zhang et al. 2012). In this interaction, aphids feed on phloem sap from their host plant  
62 excreting honeydew as waste product, a sugary-rich substance source of nutrients for the  
63 maintenance and development of ants' colonies (Stadler and Dixon 2005; Styrsky and  
64 Eubanks 2007). In return, ants protect aphids from their natural enemies and reduce disease  
65 incidence by removing waste product. Consequently ant attendance can increase aphid's  
66 colony survival, individual growth rates (Zhou et al. 2015) and individual fitness (Flatt and  
67 Weisser 2000), while ants have a permanent and easily accessible source of nutrients  
68 (Buckley 1987; Styrsky and Eubanks 2007).

69  
70 Ants engaged in mutualistic interactions with aphids become more aggressive towards any  
71 competing arthropod, deterring aphids' natural enemies like coccinellids, syrphids,  
72 neuropteran larvae and aphidiid wasps, (Breton and Addicott 1992; Kaneko 2003; Renault et  
73 al. 2005; Mooney et al. 2016). As a consequence, ant attendance can enhance aphid outbreaks  
74 (LeVan and Holway 2015) and increase sap phloem extraction, leading to negative indirect  
75 effects on fruit and seed production (Canedo-Júnior et al. 2017) and seed viability (Renault et

76 al. 2005). However, ants can also have a positive effect on the host plant by reducing the  
77 abundance of other plant herbivores and so decreasing herbivore damage (Rosumek et al.  
78 2009; Styrsky and Eubanks 2010; Trager et al. 2010; Zhang et al. 2012). Therefore, the net  
79 effects of ant-aphid interactions on the host plant represent a trade-off between the direct costs  
80 of sap-feeding by aphids and the indirect benefit of ant protection against leaf-chewing  
81 herbivores (Messina 1981; Styrsky and Eubanks 2007).

82

83 The effect of ant-aphid mutualisms on host plants is also mediated by other factors such as the  
84 aphids and ants species (Clark and Singer 2018), or traits of the host plant (Heil and McKey  
85 2003). For example, plant species with small or ephemeral leaves and hard tissues are  
86 expected to bear a lower abundance and diversity of insect herbivores and consequently the  
87 potential benefits of ant protection may not compensate for (or even exacerbate) the direct  
88 damage by sap-feeding aphids (Vilela and Del-Claro 2018). Therefore, the net outcome may  
89 have a negative impact on plant growth (foliage or growing stems) or reproductive  
90 performance (fruit and seed production) (Zvereva et al. 2010).

91

92 In this study, we investigated whether the interaction between ants and aphids affects host  
93 plant reproduction through direct and indirect effects on *Retama sphaerocarpa* (L.) Boiss in a  
94 Mediterranean grassland in the Iberian Peninsula. *Retama sphaerocarpa* is a leguminous  
95 shrub relatively common in degraded and abandoned semi-arid Mediterranean lands. This  
96 shrub can potentially host four aphid species, which are also found in other Fabaceae species  
97 (Holman 2009), that can engage in mutualistic interactions with several ant species (Nieto et  
98 al. 2002). The cost of these ant-aphid interactions for the host plant will depend on the net  
99 benefit of patrolling ants on herbivory reduction. The assemblage of insect herbivores in these  
100 shrubs tend to show low diversity and be dominated by specialist insects (Megías et al. 2011).

101 This may be partly due to traits characteristic of this plant species, such as the production of  
102 ephemeral leaves that last few days and the hard, photosynthetic stems that contain chemical  
103 compounds (López et al. 2001). To test whether the interaction between ants and aphids  
104 indirectly benefit *R. sphaerocarpa* by reducing herbivores, we experimentally manipulated  
105 the presence of ants and aphids on individual plants during the fruiting period. We  
106 specifically address whether the presence or absence of ant-aphid interactions affects (1)  
107 aphid abundance, (2) the abundance of aphid predators and plant herbivores, (3) damage by  
108 arthropods to mature fruits, and (4) fruit and seed production.

109

110

## 111 **Materials and methods**

### 112 *Study area*

113 Fieldwork was conducted during July and August 2016 in Valdelosielos (40°35' N, 3°44'  
114 W) near Tres Cantos, central Spain, at 700-750 m.a.s.l. The continental Mediterranean climate  
115 has an average annual precipitation between 400-600 mm with a mean annual temperature of  
116 15-17 °C (Ninyerola et al. 2005). The area is characterised by acidic soils and gentle slopes  
117 dominated by grasslands, sparse shrubs, mainly *R. sphaerocarpa* and *Juniperus communis*,  
118 and scattered *Quercus rotundifolia* trees.

119

### 120 *Study organisms*

121 *Retama sphaerocarpa* is a xerophytic, leguminous shrub widely distributed throughout the  
122 Iberian Peninsula and North Africa. It is a pioneer species and a major structural component  
123 of the native plant communities in many abandoned fields. This almost leafless shrub has  
124 multiple photosynthetic branches (Pugnaire et al. 1996) and can grow up to 3 m high (Fig. 1a,  
125 c). Flowering and fruiting take place from April to July, producing indehiscent pods

126 containing 1-3 seeds per fruit (Peñas 2009). *Retama sphaerocarpa* is a host for four aphid  
127 species: *Acyrtosiphon pisum* Harris, *Aphis fabae* Scopoli, *Aphis craccivora* Koch and *Aphis*  
128 *cytisorum* Hartig (Holman 2009). However, only *A. craccivora* or/and *A. cytisorum* (Fig. 1b,  
129 c) were found in the study area. Both species are worldwide distributed being more common  
130 in temperate, tropical and Mediterranean regions in association with species in Fabaceae  
131 family (Szentesi and Wink 1991). Both *A. craccivora* and *A. cytisorum* are members of a  
132 closely related complex of grey-black aphids that are indistinguishable in the field (N. Pérez,  
133 pers. com.). Both species lay their eggs at the base of plants from where females will emerge  
134 and start their life cycle. Young colonies are found on the growing parts of the plant (Szentesi  
135 and Wink 1991).

136

137 Both species are facultative trophobionts associated with different ant species from the  
138 subfamilies Formicinae, Myrmicinae and Dolichoderinae (Nieto et al. 2002). In the studied  
139 plants three ant species were found tending aphids (Fig. 1 b, c): *Crematogaster auberti* Emery  
140 (Myrmicinae), *Camponotus foreli* Emery and *Camponotus aethiops* Latreille (Formicinae).  
141 Common potential predators of these aphid species are coccinellids (Coleoptera), syrphids and  
142 cecydomids (Diptera), spiders (Araneae) and earwigs (Dermaptera) (Pinol et al. 2009).

143

#### 144 *Field experiments*

145 In an area of over 10 ha, we chose 20 plants of equivalent size, no signs of damage or brown  
146 parts in their branches and with similar level of aphid infection, as estimated visually. In each  
147 plant, we selected three branches between 40-60 cm long and randomly assigned one of three  
148 different treatments: (a) total exclusion: aphids and ants were manually removed from  
149 branches; (b) ant exclusion: ants were manually removed and aphids remained on branches;  
150 and (c) control: aphid colonies were tended by ants. We used sticky bands, with flanges on

151 both ends, as barriers for ant exclusion. Potential plant bridges to branches were trimmed to  
152 prevent ant access. In addition, we used similar bands tied with flanges, but without sticky  
153 resin, on control branches to simulate manipulation. Branches were checked two or three  
154 times per week to ensure the effectiveness of ant-exclusion treatments and any aphid  
155 colonising total exclusions was removed. We note that the use of branches as experimental  
156 treatments may overestimate to some extent the short-term effect of ant-aphid interactions on  
157 branches compared to similar treatments covering the whole plant, because woody plants are  
158 able to partially compensate herbivore damage in the next growing seasons (Obeso 1993; but  
159 see Zhang et al. 2015, for consistent effects between the branch and plant scales).

160

#### 161 *Effect of ant-tending on aphid abundance*

162 To evaluate the effect of ant-tending on aphid abundance, we monitored ants and aphids on  
163 ant-excluded and control branches twice a week, between 8:00 a.m. and 12:00 p.m., for five  
164 consecutive weeks until no aphids were found on branches (a total of 10 recording dates). On  
165 each date, we counted the number of ants on control branches and took digital photographs of  
166 ant-excluded and control branches to later estimate the number of aphids (Supplementary  
167 Fig.1). Aphids were counted by marking them individually in the digital photographs using  
168 the multi-point tool in the ImageJ software (Abràmoff et al. 2004), thus avoiding double  
169 counting.

170

#### 171 *Effect of ant-tending on the arthropod community*

172 To test the effect of ant-aphid interaction on the host plant's arthropod community, we  
173 recorded the arthropods presence on experimental branches on each sampling date.  
174 Arthropods were visually identified up to order or family level or collected from other  
175 branches and preserved in ethanol (70%) for later identification. The recorded arthropods



176 were classified into three broad functional groups: (1) aphid predators: Coccinellidae  
177 (Coleoptera; pupae, larvae and adults) (Fig. 1d) and Araneae; (2) plant herbivores:  
178 Lepidoptera (larvae) (Fig. 1e), Caelifera (Orthoptera), Membracidae (Homoptera) (Fig. 1f);  
179 and (3) others: Dermestidae and Erotylidae (Coleoptera), mainly fungus feeders,  
180 saprophagous and scavengers (Robertson et al. 2004).

181

### 182 *Effect of ant-aphid interactions on host plant reproduction*

183 To evaluate the effect of ant-aphid interactions on the reproductive output of *R.*  
184 *sphaerocarpa*, we collected and counted the total number of fruits from experimental  
185 branches following aphid death and once fruits were fully ripened. We examined all fruits  
186 under a dissecting microscope to detect signs of arthropod attack (holes, gnawed areas) to  
187 calculate the proportion of damaged fruits for each branch. We found an unidentified moth  
188 (Lepidoptera) in our fruit samples that likely attacked fruits and damaged seeds. We randomly  
189 selected 10 undamaged fruits (when available) from each experimental branch and measured  
190 fruit and seed mass to the nearest 0.01 mg with a digital scale after fruits had been oven-dried  
191 for 24 h at 60 °C. Pericarp mass was calculated by subtracting total seed mass from fruit mass  
192 for each measured fruit. We also counted the number of seeds per fruit and calculated seed  
193 output as the total number of seeds produced in undamaged fruits for each experimental  
194 branch.

195

### 196 *Statistical analyses*

197 All analyses were performed in R version 3.3.2 (R Core Development Team). To test whether  
198 temporal variations in aphid abundance differed when aphids are tended or unattended by  
199 ants, we fitted a generalised additive mixed model (GAMM) with plants as the random  
200 variable, due to the non-linear temporal variation in aphid abundance, using the *gam4*

201 package (Wood and Scheipl, 2017). Data on aphid abundances were overdispersed and non-  
202 normally distributed, so we used a negative binomial error distribution (Bolker et al. 2009).  
203 We included treatment (ants present or excluded), time in days and the interaction between  
204 treatment and time as fixed factors, and the initial number of aphids (standardised to zero  
205 mean and unit variance) as a covariate. For branches with ants tending aphids, we also  
206 assessed temporal variations in ant abundance in relation to aphid abundance, fitting a  
207 GAMM with a negative binomial error distribution. The number of ants counted per branch  
208 on each date was included together with time in days as fixed factors in the model, number of  
209 aphids as response variable and plant as random factor.

210

211 To assess whether the presence of ants affected the abundance of aphid predators  
212 (Coccinellidae) and plant herbivores we used generalised linear mixed models (GLMM) using  
213 the *lme4* package (Bates et al. 2015). Models were fitted to the negative binomial distribution  
214 due to a high proportion of zeroes (Bolker et al. 2009). Plant herbivores were tested separately  
215 for Lepidoptera (caterpillars; leaf-chewing herbivores) and Membracidae (treehoppers; sap-  
216 feeders that also produce honeydew and could potentially interact as trophobionts with ants).  
217 We also tested both groups together with Caelifera grasshoppers as plant herbivores. As the  
218 number of predators and herbivores recorded on each date were relatively low, we used the  
219 cumulative number of predators or herbivores counted during all recording dates as the  
220 response variables. Experimental treatment (ants present or excluded) was included as a fixed  
221 factor and plant as a random factor. We also included the maximum number of aphids  
222 (standardised to zero mean and unit variance) registered on the branch as a covariate in the  
223 model for aphid predators to account for the potential correlation between aphid and predator  
224 abundance (Pinol et al. 2009), and the interaction between treatment and aphid abundance.

225 We used linear and generalised mixed models to test for the effect of the presence of aphids  
226 and tending ants, the exclusion of ants, or the exclusion of both aphids and ants (experimental  
227 treatments) on plant reproduction. GLMMs were used to test for differences in the number of  
228 fruits produced per branch (Poisson error), the proportion of damaged fruits (binomial error),  
229 and seed output (Poisson error; after rounding values to the nearest integer). Pericarp mass,  
230 total seed mass and number of seeds per fruit were normally distributed, so we used linear  
231 mixed models. All mixed models included plant as a random factor and were fitted using the  
232 *glmer* and *lmer* functions in the *lme4* (Bates et al. 2015) and the *lmerTest* packages  
233 (Kuznetsova et al. 2017). All models were simplified by sequentially removing non-  
234 significant terms and selected according to the Akaike information criterion (Crawley 2007).  
235 Some branches produced few fruits, so we just included branches that produced more than  
236 three fruits on the analyses of fruit and seed traits, and the proportion of damaged fruits ( $n =$   
237 11, 17 and 20 branches for the control, ant exclusion and ant plus aphid exclusion treatments;  
238 respectively). We measured the length and two perpendicular diameters of the experimental  
239 branches and did not find differences in branch length, area or volume among the three  
240 treatments (ANOVA, length:  $F_{2,57} = 0.22$ ,  $P = 0.801$ ; area:  $F_{2,57} = 0.32$ ,  $P = 0.727$ ; volume:  
241  $F_{2,57} = 0.37$ ,  $P = 0.693$ ), so we did not correct for sampling effort.

242

## 243 **Results**

### 244 *Effect of ant-tending on aphid abundance*

245 The abundance of aphids on branches was significantly higher in the presence of ants  
246 (GAMM,  $Z = -13.55$ ;  $P < 0.001$ ). Aphid abundance was 3.4 times greater on control branches  
247 (mean  $\pm$  SE:  $790.5 \pm 76.1$  aphids,  $n = 20$  plants) than the abundance in ant-excluded branches  
248 ( $229.2 \pm 37.0$  aphids,  $n = 20$  plants; Fig. 2). There was a marked increase in the number of

249 aphids during the first ten days when tended by ants, reaching the highest average abundance  
250 after 14 days ( $1285 \pm 321$  aphids per branch; Fig. 2). Aphid numbers remained stable for over  
251 four more days and dropped significantly during the following 15 days (Fig. 2). Aphids on  
252 branches where ants were excluded increased moderately until reaching a maximum after 11  
253 days (average abundance:  $464 \pm 210$  aphids per branch; Fig. 2), after which aphids began to  
254 decline steadily for 10 days until individuals dispersed or died out (Fig. 2). Overall, ant-  
255 tended aphids survived 12 more days than those on ant-excluded branches before populations  
256 crashed (Fig. 2). For control branches, with ants tending aphids, the number of ants was  
257 positively correlated with the abundance of aphids (GAMM,  $Z = 2.44$ ,  $P < 0.015$ ), although  
258 the number of ants on branches decreased over time (Fig. 3).

259

#### 260 *Effect of ant-tending on the arthropod community*

261 During the experiment, we recorded 629 arthropods from eight different taxa: 72.3% ( $n =$   
262 455) corresponded to Coccinellidae at different developmental stages (i.e., pupa, larva and  
263 adult); 24.8% ( $n = 156$ ) were plant herbivores, with lepidopteran larvae ( $n = 104$ ) and adult  
264 membracids ( $n = 46$ ) being the most abundant, while Caelifera (grasshoppers) were rare ( $n =$   
265 6); and the remaining 2.9% were Coleoptera ( $n = 17$ ) and Araneae ( $n = 1$ ) (Table 1).

266 Coccinellidae abundance was lower in the presence of ants compared to that in ant-excluded  
267 branches (Table 1; GLMM,  $Z = 2.62$   $P = 0.002$ ). We found higher coccinellid abundance in  
268 branches with more aphids (GLMM,  $Z = 2.41$ ;  $P = 0.016$ ), however, no significant interaction  
269 between treatment and aphid abundance was found (GLMM,  $Z = 1.12$ ,  $P = 0.263$ ).

270 The number of lepidopteran larvae and membracids recorded were relatively low and did not  
271 differ significantly between treatments (Table 1, GLMM,  $Z < 1.47$ ,  $P > 0.141$ ; for both  
272 groups). Results were similar when grouping lepidopteran larvae, membracids and Caelifera  
273 grasshoppers as plant herbivores (GLMM,  $Z = 0.78$ ,  $P = 0.431$ ).

274

275 *Effect of ant-aphid interaction on host plant reproduction*

276 The number of fruits produced was highest for total exclusion branches, followed by ant-  
277 excluded branches and control branches (Table 2, Fig. 4a). There was a greater proportion of  
278 damaged fruits in control branches ( $35.2 \pm 8.9\%$ ,  $n = 11$ ) than in ant exclusion branches ( $22.9$   
279  $\pm 7.0\%$ ,  $n = 17$ ) and total exclusion branches ( $13.7 \pm 2.4\%$ ,  $n = 20$ ; Table 2, Fig. 4b). Fruits  
280 produced on each experimental treatment did not differ in pericarp mass (control:  $37.4 \pm 3.7$   
281 mg,  $n = 11$ ; ant exclusion:  $50.2 \pm 8.1$  mg,  $n = 17$ ; total exclusion:  $34.8 \pm 2.6$  mg,  $n = 20$ ; Table  
282 2) or total seed mass ( $60.1 \pm 7.2$  mg,  $n = 11$ ;  $68.2 \pm 3.9$  mg,  $n = 17$ ;  $70.6 \pm 3.4$  mg,  $n = 20$ ;  
283 respectively; Table 2), although fruits in ant-excluded branches had fewer seeds than those in  
284 control and total exclusion branches (Table 2, Fig. 4c). Overall, the estimated seed output for  
285 all branches was lowest in control branches ( $37.3 \pm 20.9$ ,  $n = 20$ ), increasing in ant-excluded  
286 branches ( $55.2 \pm 12.5$ ,  $n = 20$ ) and total exclusion branches ( $195.1 \pm 32.3$ ,  $n = 20$ ) (Table 2;  
287 Fig. 4d).

288

289 **Discussion**

290 Our results indicate that the presence of ants enhanced aphid abundance and reduced the  
291 incidence of aphid predators, but had no influence on other plant herbivores, resulting in a net  
292 negative effect for the host plant in terms of its reproductive output. Nevertheless, aphids in  
293 the absence of ants also decrease fruit and seed production in *R. sphaerocarpa*. Furthermore,  
294 arthropods attacking fruits during the predispersal phase increased the impact on *R.*  
295 *sphaerocarpa* reproduction with a greater proportion of damaged fruits on the already smaller  
296 crop sizes in treatments with aphids. Our results highlight the relevance of conducting multi-  
297 trophic approaches in order to elucidate ant-aphid mutualism outcomes within a community  
298 context.

299

300 *Effects of ants on aphid population*

301 Aphids were 3.4 times more abundant in the presence of ants and persisted longer, agreeing  
302 with previous studies that showed how ants can enhance aphid reproductive rate, survival and  
303 longevity (Flatt and Weisser 2000; Hosseini et al. 2017; Canedo-Júnior et al. 2018).

304 Unattended aphid populations collapsed within the first 20 days, whilst most of ant-attended  
305 aphids survived until the end of the experiment (i.e. 34 days). This finding supports the idea  
306 that ant attendance enhances the persistence of aphid colonies probably due to the increased  
307 defence against predators and perhaps parasitoids (Stadler and Dixon 1999). Ant attendance  
308 decreased over time, presumably due to changes in feeding preferences by ants (Offenberg  
309 2001), variation in the attractiveness of honeydew relative to sugar composition (Yao 2014)  
310 or changes in the nutritional requirements of ants (Ivens 2015).

311

312 *Effects of ant-aphid mutualism on aphid predators and plant herbivores*

313 Our results showed that the presence of ants significantly decreased the number of ladybirds,  
314 the main aphid predator detected in our study system, by deterring or preying on them. As a  
315 result, aphids were much more abundant and persisted longer in control than in ant-excluded  
316 treatments. Ladybirds are important predators of aphids, coccids, aleyrodids and psyllids  
317 (Sternorrhyncha suborder), and meta-analyses have shown the strong effects of ants on the  
318 abundance of aphid predators (Rosumek et al. 2009; Trager et al. 2010; Zhang et al. 2012).

319 We found relatively low and similar number of non-aphid herbivores between treatments, so  
320 the presence of ants did not affect the abundance of plant herbivores. *Retama sphaerocarpa*  
321 produces small and ephemeral leaves (Pugnaire et al. 1996), so it is leafless most of the time,  
322 and the photosynthetic branches contain defensive chemical compounds (Martín-Cordero et  
323 al. 1997), that act as a defence against generalist herbivores (Megías et al. 2011). The only

324 plant parts attractive to plant herbivores appeared to be buds, developing fruits and terminal  
325 branches, where aphids were densely clumped. Because the presence of ants favoured aphids  
326 but did not reduce the abundance of other plant herbivores, the cost of sap-feeding by aphids  
327 was not presumably compensated by a reduction in herbivory and thus the net outcome was  
328 negative for the host plant.

329

### 330 *Consequences of ant-aphid mutualism on the host plant reproductive output*

331 Fruit production was severely reduced in control branches, with 33% and 83% less seeds  
332 than in ant-exclusion and total exclusion branches respectively (Fig. 4). Even so, the impact  
333 of non-attended aphids on plant reproduction was significant (Snow and Stanton 1988). Fruit  
334 abortion during development seemed the main cause of fruit loss due to aphids feeding  
335 directly on growing fruits (Fig. 4c) or indirectly when feeding on terminal branches. For  
336 example, fruit set, estimated from initial growing to ripening, was 2.2-fold higher for  
337 branches with aphids present and ants excluded compared to branches with ants attending  
338 aphids.

339

340 The consequences of ant-aphid mutualism on the abundance of leaf-chewing herbivores have  
341 been more commonly addressed than those on plant reproductive performance, especially in  
342 temperate regions (Trager et al. 2010; Zhang et al. 2012). Our results are in line with studies  
343 showing that ant-aphid mutualism negatively affects plant reproductive performance in a  
344 community context, by decreasing the number of fruits, seeds, seed mass or seed viability  
345 (Renault et al. 2005; LeVan and Holway 2015; Canedo-Júnior et al. 2017; Ibarra-Isassi and  
346 Oliveira 2018). For example, Canedo-Júnior et al. (2017) found higher density and longevity  
347 of aphids in the presence of ants, increasing plant damage by aphids that, together with  
348 damages by other herbivores, reduced the amount of energy invested by the host plant on

349 reproduction. Other studies reported that negative effects on seed production resulted from  
350 indirect interactions during previous stages of reproduction, as the presence of ants tending  
351 honeydew-producing hemipterans disrupted insect pollination (Levan and Holway 2015;  
352 Ibarra-Isassi and Oliveira 2018). However, other studies suggested that ant-aphid interactions  
353 have positive effects on the host plant by reducing herbivory on the leaves and flowers (Del-  
354 Claro et al. 2006; Styrsky and Eubanks 2010) and improving the production of above ground  
355 biomass (Hosseini et al. 2017). All this shows the context-dependence of the cascading  
356 effects of ant-aphid interactions (Chamberlain et al. 2014). The identity of interacting species,  
357 the strength of the mutualistic-agonistic interactions, herbivory levels or plant characteristics  
358 would mediate the outcome of these multitrophic interactions (Heil and McKey 2003; Schuldt  
359 et al. 2017).

360

361 Seed output was severely affected by ant-aphid mutualism, but also by aphids alone compared  
362 to the exclusion of aphids and ants. Thus, the number of fruits produced on the experimental  
363 branches was the main difference in reproductive performance among treatments. We did not  
364 find differences in pericarp and total seed mass for the fruits produced in each treatment. We  
365 found that total exclusion branches produced fruits with less seeds. However, most fruits  
366 produced by *R. sphaerocarpa* have one seed and sample size differs among treatments due to  
367 lower crops when aphids are present, so whether these variations in seed number are  
368 biologically meaningful remains unclear.

369

370 We found that the proportion of damaged fruits to the total number of fruits produced was  
371 lower in total exclusions, followed by ant exclusions and control branches. These variations in  
372 fruit damage between branches likely resulted from differences in fruit availability within the  
373 same plant, branches with less fruits were proportionally more damaged than branches with



374 more fruits. Consequently, the presence of ants tending aphids resulted in an average of 88%  
375 reduction in fruit production between total exclusion and control branches. As noted earlier,  
376 even though the detrimental effect of aphids on *R. sphaerocarpa* may be somewhat  
377 overestimated in the long-term, short-term figures are reasonable given the rarity of  
378 compensatory responses of plants to sap-feeders (Zvereva et al. 2010).

379

380 To conclude, this study supports that investigating ant-aphid mutualisms within a community  
381 context is important if we are to understand the factors mediating the effects of these  
382 interactions on plant fitness; e.g., host plant characteristics can be determinant for the final  
383 outcome of the mutualistic interactions. Furthermore, given that ant-aphid mutualisms are  
384 widespread in nature, future research should pay more attention to these multitrophic  
385 interactions, as global change can jeopardize ecosystem services provided by these  
386 communities, something that could be crucial for nitrogen-fixing pioneer species in degraded  
387 semi-arid Mediterranean areas such as *R. sphaerocarpa*.

388

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