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Abstract

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

Keywords (separated by '-'): Ant–aphid mutualism - Herbivory - Indirect interactions - *Retama sphaerocarpa* - Seed production - Top-down effects

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

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

Keywords Ant–aphid mutualism · Herbivory · Indirect interactions · *Retama sphaerocarpa* · Seed production · Top-down effects

Introduction

Trophic interactions are key determinants of ecosystem functioning, population dynamics and community structure (Ings et al. 2009). Many outcomes of these interactions are difficult to anticipate as they include mutualistic and antagonistic species interactions that cannot be studied by pairwise interactions alone (Reiss AQ1

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et al. 2009). More complex approaches are needed to integrate positive and negative links as well as direct and indirect effects among several trophic levels (Clark et al. 2016; Seibold et al. 2018). Individual plants can host a wide range of arthropods that are engaged in multiple interaction types, and each interaction may have different impacts on host plant performance (Ando et al. 2017). A keystone interaction affecting several ecological processes in plants is the mutualism between ants and aphids (Zhang et al. 2012). In this interaction, aphids feed on phloem sap from their host plant excreting honeydew as waste product, a sugary-rich substance source of nutrients for the maintenance and development of ants' colonies (Stadler and Dixon 2005; Styrsky and Eubanks 2007). In return, ants protect aphids from their natural enemies and reduce disease incidence by removing waste product. Consequently ant attendance can increase aphid's colony survival, individual growth rates (Zhou et al. 2015) and individual fitness (Flatt and Weisser 2000), while ants have a permanent and easily accessible source of nutrients (Buckley 1987; Styrsky and Eubanks 2007).

Ants engaged in mutualistic interactions with aphids become more aggressive towards any competing arthropod, deterring aphids' natural enemies such as coccinellids, syrphids, neuropteran larvae and aphidiid wasps, (Breton and Addicott 1992; Kaneko 2003; Renault et al. 2005; Mooney et al. 2016). As a consequence, ant attendance can enhance aphid outbreaks (LeVan and Holway 2015) and increase sap phloem extraction, leading to negative indirect effects on fruit and seed production (Canedo-Júnior et al. 2017) and seed viability (Renault et al. 2005). However, ants can also have a positive effect on the host plant by reducing the abundance of other plant herbivores and so decreasing herbivore damage (Rosumek et al. 2009; Styrsky and Eubanks 2010; Trager et al. 2010; Zhang et al. 2012). Therefore, the net effects of ant–aphid interactions on the host plant represent a trade-off between the direct costs of sap-feeding by aphids and the indirect benefit of ant protection against leaf-chewing herbivores (Messina 1981; Styrsky and Eubanks 2007).

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

In this study, we investigated whether the interaction between ants and aphids affects host plant reproduction through direct and indirect effects on *Retama sphaerocarpa* (L.) Boiss in a Mediterranean grassland in the Iberian Peninsula. *Retama sphaerocarpa* is a leguminous shrub relatively common in degraded and abandoned semi-arid Mediterranean lands. This shrub can potentially host four aphid species, which are also found in other Fabaceae species (Holman 2009), that can engage in mutualistic interactions with several ant species (Nieto et al. 2002). The cost of these ant–aphid interactions for the host plant will depend on the net benefit of patrolling ants on herbivory reduction. The assemblage of insect herbivores in these shrubs tends to show low diversity and be dominated by specialist insects (Megías et al. 2011). This may be partly due to traits characteristic of this plant species, such as the production of ephemeral leaves that last few days and the hard, photosynthetic stems that contain chemical compounds (López et al. 2001). To test whether the interaction between ants and aphids indirectly benefit *R. sphaerocarpa* by reducing herbivores, we experimentally manipulated the presence of ants and aphids on individual plants during the fruiting period. We specifically address whether the presence or absence of ant–aphid interactions affects (1) aphid abundance, (2) the abundance of aphid predators and plant herbivores, (3) damage by arthropods to mature fruits, and (4) fruit and seed production.

Materials and methods

Study area

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

Study organisms

Retama sphaerocarpa is a xerophytic, leguminous shrub widely distributed throughout the Iberian Peninsula and North Africa. It is a pioneer species and a major structural component of the native plant communities in many abandoned fields. This almost leafless shrub has multiple photosynthetic branches (Pugnaire et al. 1996) and can grow up to 3 m high (Fig. 1a, c). Flowering and fruiting take place from April to July, producing indehiscent pods containing 1–3 seeds per fruit (Peñas 2009). *Retama sphaerocarpa* is a host for four aphid species: *Acyrthosiphon pisum* Harris, *Aphis fabae* Scopoli, *Aphis craccivora* Koch and *Aphis cytisorum* Hartig (Holman 2009). However, only *A. craccivora* or/and *A. cytisorum* (Fig. 1b, c) were found in the study area. Both species are worldwide distributed being more common in temperate, tropical and Mediterranean regions in association with species in Fabaceae family (Szentesi and Wink 1991). Both *A. craccivora* and *A. cytisorum* are members of a closely related complex of grey–black aphids that are indistinguishable in the field (N. Pérez, pers. com.). Both species lay their eggs at the base of plants from where females will emerge and start their life cycle. Young colonies are found on the growing parts of the plant (Szentesi and Wink 1991).

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

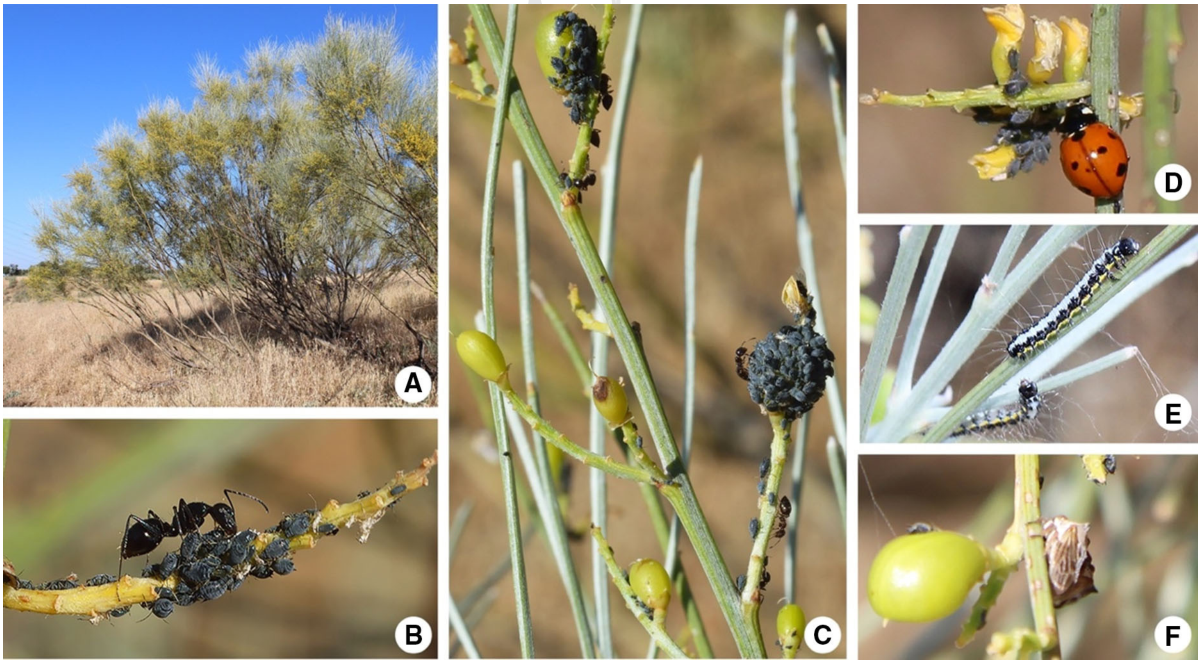

Fig. 1 **a** Individual of *Retama sphaerocarpa* in the study area. **b** Aphids tended by an ant on a twig of *R. sphaerocarpa*. **c** Aphids on branches and fruits of *R. sphaerocarpa* being tended by ants. **d** Ladybird depredating on aphids. **e** Caterpillars on *R. sphaerocarpa* branches. **f** Treehopper and a developing fruit of *R. sphaerocarpa*

Field experiments

In an area of over 10 ha, we chose 20 plants of equivalent size, no signs of damage or brown parts in their branches and with similar level of aphid infection, as estimated visually. In each plant, we selected three branches between 40 and 60 cm long and randomly assigned one of three different treatments: (a) total exclusion: aphids and ants were manually removed from branches; (b) ant exclusion: ants were manually removed and aphids remained on branches; and (c) control: aphid colonies were tended by ants. We used sticky bands, with flanges on both ends, as barriers for ant exclusion. Potential plant bridges to branches were trimmed to prevent ant access. In addition, we used similar bands tied with flanges, but without sticky resin, on control branches to simulate manipulation. Branches were checked two or three times per week to ensure the effectiveness of ant-exclusion treatments and any aphid colonising total exclusions was removed. We note that the use of branches as experimental treatments may overestimate to some extent the short-term effect of ant–aphid interactions on branches compared to similar treatments covering the whole plant, because woody plants are able to partially compensate herbivore damage in the next growing seasons (Obeso 1993; but see Zhang et al. 2015, for consistent effects between the branch and plant scales).

Effect of ant tending on aphid abundance

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

Effect of ant tending on the arthropod community

To test the effect of ant–aphid interaction on the host plant's arthropod community, we recorded the arthropods presence on experimental branches on each sampling date. Arthropods were visually identified up to order or family level or collected from other branches and preserved in ethanol (70%) for later identification. The recorded arthropods were classified into three broad functional groups: (1) aphid predators: Coccinellidae (Coleoptera; pupae, larvae and adults) (Fig. 1d) and Araneae; (2) plant herbivores: Lepidoptera (larvae) (Fig. 1e), Caelifera (Orthoptera), Membracidae (Homoptera) (Fig. 1f); and (3) others: Dermestidae and Erotylidae (Coleoptera), mainly fungus feeders, saprophagous and scavengers (Robertson et al. 2004).

Effect of ant–aphid interactions on host plant reproduction

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

Statistical analyses

All analyses were performed in R version 3.3.2 (R Core Development Team). To test whether temporal variations in aphid abundance differed when aphids are tended or unattended by ants, we fitted a generalised additive mixed model (GAMM) with plants as

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the random variable, due to the non-linear temporal variation in aphid abundance, using the *gamm4* package (Wood and Scheipl 2017). Data on aphid abundances were overdispersed and non-normally distributed, so we used a negative binomial error distribution (Bolker et al. 2009). We included treatment (ants present or excluded), time in days and the interaction between treatment and time as fixed factors, and the initial number of aphids (standardised to zero mean and unit variance) as a covariate. For branches with ants tending aphids, we also assessed temporal variations in ant abundance in relation to aphid abundance, fitting a GAMM with a negative binomial error distribution. The number of ants counted per branch on each date was included together with time in days as fixed factors in the model, number of aphids as response variable and plant as random factor.

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

We used linear and generalised mixed models to test for the effect of the presence of aphids and tending ants, the exclusion of ants, or the exclusion of both aphids and ants (experimental treatments) on plant reproduction. GLMMs were used to test for differences in the number of fruits produced per branch (Poisson error), the proportion of damaged fruits (binomial error), and seed output (Poisson error; after rounding values to the nearest integer). Pericarp mass, total seed mass and number of seeds per fruit were normally distributed, so we used linear mixed models. All mixed models included plant as a random factor and were fitted using the *glmer* and *lmer* functions in the *lme4* (Bates et al. 2015) and the *lmerTest* packages (Kuznetsova et al. 2017). All models were simplified by sequentially removing non-significant terms and selected according to the Akaike information criterion (Crawley 2007).

Some branches produced few fruits, so we just included branches that produced more than three fruits on the analyses of fruit and seed traits, and the proportion of damaged fruits ($n = 11$, 17 and 20 branches for the control, ant-exclusion and ant plus aphid exclusion treatments; respectively). We measured the length and two perpendicular diameters of the experimental branches and did not find differences in branch length, area or volume among the three treatments (ANOVA, length: $F_{2,57} = 0.22$, $P = 0.801$; area: $F_{2,57} = 0.32$, $P = 0.727$; volume: $F_{2,57} = 0.37$, $P = 0.693$), so we did not correct for sampling effort.

Results

Effect of ant tending on aphid abundance

The abundance of aphids on branches was significantly higher in the presence of ants (GAMM, $Z = -13.55$; $P < 0.001$). Aphid abundance was 3.4 times greater on control branches (mean ± SE: 790.5 ± 76.1 aphids, $n = 20$ plants) than the abundance in ant-excluded branches (229.2 ± 37.0 aphids, $n = 20$ plants; Fig. 2). There was a marked increase in the number of aphids during the first ten days when tended by ants, reaching the highest average abundance after 14 days (1285 ± 321 aphids per branch; Fig. 2). Aphid numbers remained stable for over four more days and dropped significantly during the following 15 days (Fig. 2). Aphids on branches where ants were excluded increased moderately until reaching a maximum after 11 days (average abundance: 464 ± 210 aphids per branch; Fig. 2), after which aphids began to decline steadily for 10 days until individuals dispersed or died out (Fig. 2). Overall, ant-tended aphids survived 12 more days than those on

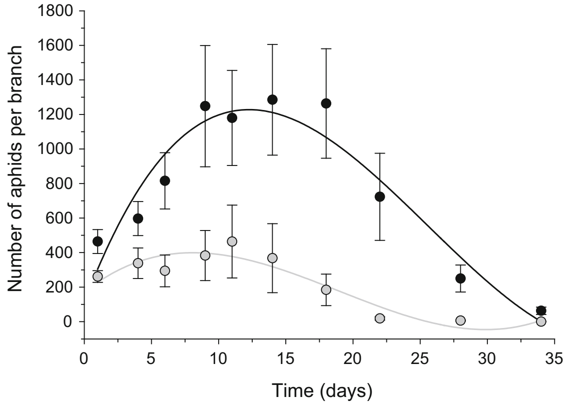

Fig. 2 Temporal variation in the number of aphids (± SE) on branches of 20 *Retama sphaerocarpa* shrubs with aphid-tending ants present (control; black) or excluded (ant exclusion; grey) during the duration of the experiment. The curves represent a cubic regression fit to the data for each treatment

ant-excluded branches before populations crashed (Fig. 2). For control branches, with ants tending aphids, the number of ants was positively correlated with the abundance of aphids (GAMM, $Z = 2.44$, $P < 0.015$), although the number of ants on branches decreased over time (Fig. 3).

Effect of ant tending on the arthropod community

During the experiment, we recorded 629 arthropods from eight different taxa: 72.3% ($n = 455$) corresponded to Coccinellidae at different developmental stages (i.e., pupa, larva and adult); 24.8% ($n = 156$) were plant herbivores, with lepidopteran larvae ($n = 104$) and adult membracids ($n = 46$) being the most abundant, while Caelifera (grasshoppers) were rare ($n = 6$); and the remaining 2.9% were Coleoptera ($n = 17$) and Araneae ($n = 1$) (Table 1). Coccinellidae abundance was lower in the presence of ants compared to that in ant-excluded branches (Table 1; GLMM, $Z = 2.62$ $P = 0.002$). We found higher coccinellid abundance in branches with more aphids (GLMM, $Z = 2.41$; $P = 0.016$), however, no significant interaction between treatment and aphid abundance was found (GLMM, $Z = 1.12$, $P = 0.263$).

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

Effect of ant–aphid interaction on host plant reproduction

The number of fruits produced was highest for total exclusion branches, followed by ant-excluded branches and control branches (Table 2, Fig. 4a). There was a greater proportion of damaged fruits in

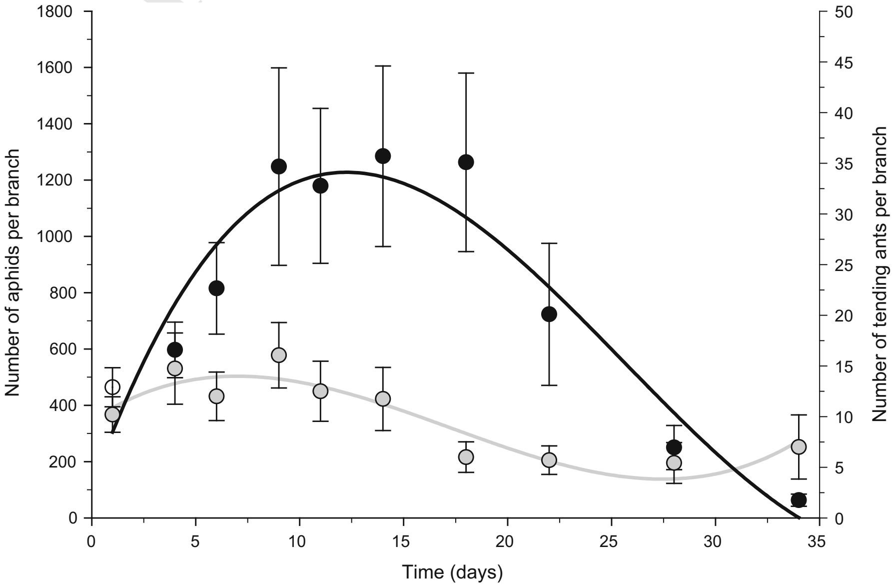

Fig. 3 Temporal variation in the number of aphids (± SE) (black) and tending ants (grey) on 20 branches of *Retama sphaerocarpa* with both species present during the duration of the experiment. The curves represent a cubic regression fit to the data for the abundance of each species. Note the different scales for each ordinate axis

Table 1 Number of arthropods (± 1 SE) assigned to three broad functional groups recorded on branches of *Retama sphaerocarpa* with aphids and tending ants (control) or branches where ants were experimentally excluded (ant exclusion) (*n* = 20 branches for each treatment)

	Experimental treatment	
	Control	Ant exclusion
Aphid predators		
Coccinellidae	7.95 ± 0.58	14.80 ± 0.97
Araneae	0.00 ± 0.00	0.05 ± 0.01
Plant herbivores		
Lepidoptera	2.35 ± 0.28	2.85 ± 0.35
Membracidae	0.85 ± 0.10	1.45 ± 0.11
Caelifera	0.15 ± 0.02	0.15 ± 0.03
Others		
Coleoptera	0.30 ± 0.05	0.55 ± 0.05

control branches (35.2 ± 8.9%, *n* = 11) than in ant-exclusion branches (22.9 ± 7.0%, *n* = 17) and total exclusion branches (13.7 ± 2.4%, *n* = 20; Table 2, Fig. 4b). Fruits produced on each experimental treatment did not differ in pericarp mass (control: 37.4 ± 3.7 mg, *n* = 11; ant exclusion: 50.2 ± 8.1 mg, *n* = 17; total exclusion: 34.8 ± 2.6 mg, *n* = 20; Table 2) or total seed mass (60.1 ± 7.2 mg, *n* = 11; 68.2 ± 3.9 mg, *n* = 17; 70.6 ± 3.4 mg, *n* = 20; respectively; Table 2), although fruits in ant-excluded branches had fewer seeds than those in control and total exclusion branches (Table 2, Fig. 4c). Overall, the estimated seed output for all branches was lowest in control branches (37.3 ± 20.9, *n* = 20), increasing in ant-excluded branches (55.2 ± 12.5, *n* = 20) and total exclusion branches (195.1 ± 32.3, *n* = 20) (Table 2; Fig. 4d).

Discussion

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

Table 2 Estimates from mixed models testing the effect of the presence of aphids and tending ants (control), the exclusion of ants, and the exclusion of both aphids and ants (total exclusion) on fruit production, fruit and seed characteristics, fruit damage and seed output in branches of 20 *Retama spaherocarpa* shrubs (sample size varies depending on the response variable)

Response variable	Treatment	Estimate	Z or t	*P*
Fruit production	Control (intercept)	3.300	24.550	**< 0.001**
	Ant exclusion	0.670	13.660	**< 0.001**
	Total exclusion	1.833	42.680	**< 0.001**
Fruit damage	Control (intercept)	− 1.180	− 5.614	**< 0.001**
	Ant exclusion	− 0.848	− 5.349	**< 0.001**
	Total exclusion	− 0.904	− 6.294	**< 0.001**
Pericarp mass	Control (intercept)	0.037	5.692	**< 0.001**
	Ant exclusion	0.013	1.501	0.140
	Total exclusion	− 0.003	− 0.313	0.756
Total seed mass	Control (intercept)	0.061	42.545	**< 0.001**
	Ant exclusion	0.006	1.089	0.286
	Total exclusion	0.009	1.747	0.092
Seed number	Control (intercept)	1.183	27.120	**< 0.001**
	Ant exclusion	− 0.128	− 2.453	**0.020**
	Total exclusion	− 0.063	− 1.266	0.215
Seed output	Control (intercept)	3.425	23.19	**< 0.001**
	Ant exclusion	0.392	8.27	**< 0.001**
	Total exclusion	1.653	41.42	**< 0.001**

Significant *P* values are shown in bold

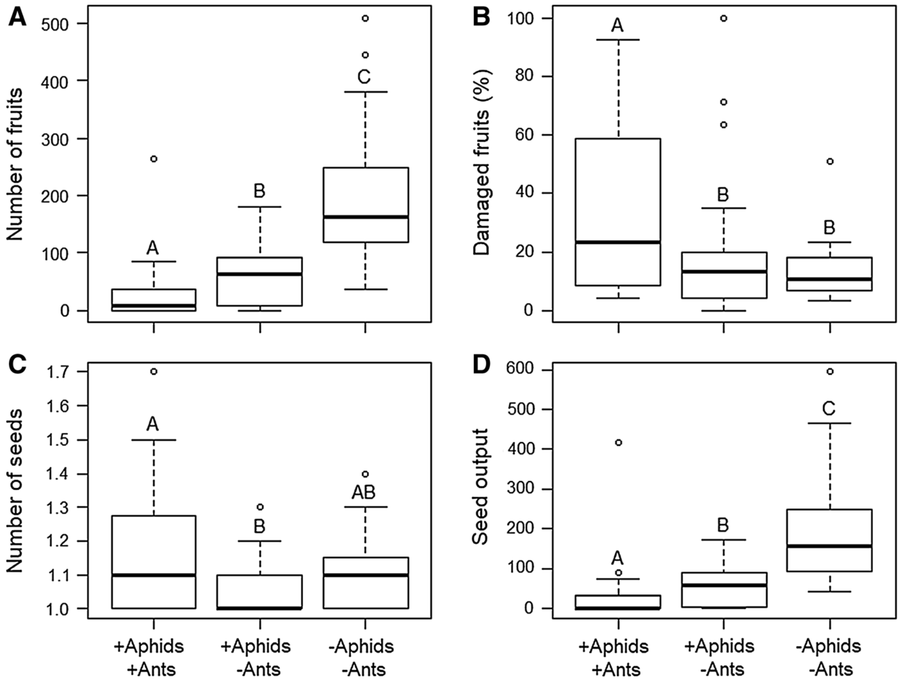

Fig. 4 **a** Number of fruits produced, **b** percentage of fruits damaged by arthropods during the predispersal phase, **c** number of seeds per fruit, and **d** seed output in branches of *Retama sphaerocarpa* with aphids tended by ants (+aphids +ants), branches with aphids and ants excluded (+aphids −ants), and branches with both aphids and ants excluded (−aphids −ants). Boxplots show the median and first and third quartiles, and whiskers indicate the ×1.5 interquartile range of data. Different letters indicate significant differences (Tukey HSD)

aphid mutualism outcomes within a community context.

Effects of ants on aphid population

Aphids were 3.4 times more abundant in the presence of ants and persisted longer, agreeing with previous studies that showed how ants can enhance aphid reproductive rate, survival and longevity (Flatt and Weisser 2000; Hosseini et al. 2017; Canedo-Júnior et al. 2018). Unattended aphid populations collapsed within the first 20 days, whilst most of ant-attended aphids survived until the end of the experiment (i.e. 34 days). This finding supports the idea that ant attendance enhances the persistence of aphid colonies probably due to the increased defence against predators and perhaps parasitoids (Stadler and Dixon 1999). Ant attendance decreased over time, presumably due to changes in feeding preferences by ants (Offenberg 2001), variation in the attractiveness of honeydew relative to sugar composition (Yao 2014) or changes in the nutritional requirements of ants (Ivens 2015).

Effects of ant–aphid mutualism on aphid predators and plant herbivores

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

We found relatively low and similar number of non-aphid herbivores between treatments, so the presence of ants did not affect the abundance of plant herbivores. *Retama sphaerocarpa* produces small and ephemeral leaves (Pugnaire et al. 1996), so it is leafless most of the time, and the photosynthetic branches contain defensive chemical compounds (Martín-Cordero et al. 1997), that act as a defence against generalist herbivores (Megías et al. 2011). The only plant parts attractive to plant herbivores appeared to be buds, developing fruits and terminal branches, where aphids were densely clumped. Because the presence of ants favoured aphids but did not reduce the abundance of other plant herbivores, the cost of sap-feeding by aphids was not presumably compensated by a reduction in herbivory and thus the net outcome was negative for the host plant.

Consequences of ant–aphid mutualism on the host plant reproductive output

Fruit production was severely reduced in control branches, with 49% and 84% less seeds than in ant-exclusion and total exclusion branches, respectively (Fig. 4). Even so, the impact of non-attended aphids on plant reproduction was significant (Snow and Stanton 1988). Fruit abortion during development seemed the main cause of fruit loss due to aphids feeding directly on growing fruits (Fig. 4c) or indirectly when feeding on terminal branches. For example, fruit set, estimated from initial growing to ripening, was 2.2-fold higher for branches with aphids present and ants excluded compared to branches with ants attending aphids.

The consequences of ant–aphid mutualism on the abundance of leaf-chewing herbivores have been more commonly addressed than those on plant reproductive performance, especially in temperate regions (Trager et al. 2010; Zhang et al. 2012). Our results are in line with studies showing that ant–aphid mutualism negatively affects plant reproductive performance in a community context, by decreasing the number of fruits, seeds, seed mass or seed viability (Renault et al. 2005; LeVan and Holway 2015; Canedo-Júnior et al. 2017; Ibarra-Isassi and Oliveira 2018). For example, Canedo-Júnior et al. (2017) found higher density and longevity of aphids in the presence of ants, increasing plant damage by aphids that, together with damages by other herbivores, reduced the amount of energy invested by the host plant on reproduction. Other studies reported that negative effects on seed production resulted from indirect interactions during previous stages of reproduction, as the presence of ants tending honeydew-producing hemipterans disrupted insect pollination (Levan and Holway 2015; Ibarra-Isassi and Oliveira 2018). However, the effects of ants on the plant performance (growth, reproduction) may not always be negative, but also neutral or positive.

Some studies found that ant–aphid interactions have positive effects on the host plant by reducing herbivory on the leaves and flowers (Del-Claro et al. 2006; Rosumek et al. 2009; Pringle et al.2017) and improving the production of above-ground biomass (Hosseini et al. 2017). Indeed, the outcome of this interaction for the plant may depend on local biotic and abiotic conditions. For example, Styrsky and Eubanks (2010) found that the indirect benefit of hosting ant–aphid interaction varied with the density of (non-aphid) herbivores. In addition, several reviews have synthesised the progress in understanding the effects of ant–aphid interactions emphasizing its context dependence (Heil and McKey 2003; Rico-Gray and Oliveira 2007; Styrsky and Eubanks 2007; Chamberlain et al. 2014). Given that studies have shown that the effect of ants on the host plant can be positive or negative depending on the context, the abundance and kind of herbivores and predators could be a key factor altering the outcome of the interaction.

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

We found that the proportion of damaged fruits to the total number of fruits produced was lower in total exclusions, followed by ant-exclusions and control branches. These variations in fruit damage between branches likely resulted from differences in fruit availability within the same plant, branches with less fruits were proportionally more damaged than branches with more fruits. Consequently, the presence of ants tending aphids resulted in an average of 88% reduction in fruit production between total exclusion and control branches. As noted earlier, even though the detrimental effect of aphids on *R. sphaerocarpa* may be somewhat overestimated in the long-term, short-term figures are reasonable given the rarity of compensatory responses of plants to sap-feeders (Zvereva et al. 2010).

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

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