

Differential ant exclusion from canopies shows contrasting top-down effects on community structure

Laia Mestre^{1,2,3} · J. Piñol^{1,2} · J. A. Barrientos¹ · X. Espadaler^{1,2}

Received: 10 February 2015 / Accepted: 30 August 2015 / Published online: 16 September 2015
© Springer-Verlag Berlin Heidelberg 2015

Abstract Predators have far-reaching effects on communities by triggering top-down trophic cascades that influence ecosystem functioning. Omnivory and intraguild interactions between predators give rise to reticulate food webs and may either strengthen or dampen trophic cascades depending on context. Disentangling the effects of multiple predator species is therefore crucial for predicting the influence of predators on community structure. We focused on ants as dominant generalist predators in arthropod communities and set up a differential ant exclusion from canopies to examine its effects on assemblage species composition and densities of five arthropod groups (psocopterans, aphids, spiders, heteropterans and beetles). We coupled a glue band with tubes allowing only the ant *Lasius grandis* to reach the canopies to isolate its effect from the rest of crawling predators (ants, earwigs) and compared it against a full exclusion and a control. *L. grandis* alone had widespread effects on assemblage species composition, with contrasting species-specific responses within groups, where some species affected by *L. grandis* presence were not further affected by the presence of the whole crawling

predator assemblage, and vice versa. Overall, *L. grandis* caused two- to threefold decreases of generalist predators and a threefold increase of aphids. However, it lacked further top-down effects on primary consumers, which only emerged when all crawling predators were present. This differential exclusion demonstrates the distinctive and widespread intraguild effects on community structure of a single ant species that contrast with the top-down effects exerted by the whole crawling predator assemblage.

Keywords Intraguild interactions · Predator effects · Taxonomic sufficiency · Top-down control · Trophic cascade

Introduction

Predators have far-reaching effects on ecological systems, from regulating prey populations and triggering top-down trophic cascades to influencing ecosystem functions like nutrient cycling (Hairston et al. 1960; Schmitz et al. 2010). Ecologists have long foregone the notion of linear trophic chains and recognize now the ubiquity of omnivory: instead of fitting into the same trophic level, predators eat each other (i.e., intraguild predation) and even feed on plants, giving rise to reticulate food webs (Polis et al. 1989; Arim and Marquet 2004; Davenport and Chalcraft 2012). Moreover, predators affect prey not only through predation but also by inducing changes in prey traits (e.g., feeding rate, dispersal), known as trait-mediated effects, which have ramifying consequences in communities (Lima and Dill 1990; Werner and Peacor 2003).

A common assumption in experimental studies is that disparate predator groups have similar effects, and are thus treated as a single functional unit in experimental

Communicated by Raphael Didham.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-015-3442-z) contains supplementary material, which is available to authorized users.

✉ Laia Mestre
laiamesar@gmail.com

¹ Universitat Autònoma Barcelona, 08193 Cerdanyola Del Vallès, Spain

² CREAM, 08193 Cerdanyola Del Vallès, Spain

³ Department of Ecology, Swedish University of Agricultural Sciences, Box 7044, 750 07 Uppsala, Sweden

manipulations (Sih et al. 1998; Schmitz 2007). Nevertheless, there is now ample evidence that interactions between predators lead to the collective effect of multiple predators being higher or lower than the sum of individual predator effects (Ives et al. 2005). Indeed, increases in predator diversity may either strengthen or dampen trophic cascades depending on context (Finke and Denno 2005; Vance-Chalcraft et al. 2007). Disentangling the effects of multiple predators is therefore crucial for predicting their influence on communities. Nevertheless, most of the research has addressed multiple predator effects on primary consumers, while the outcome of these interactions on other predators largely remains to be examined (Sitvarin and Rypstra 2014).

In arthropod communities, the study of predator effects is further complicated because most arthropod groups are hyperdiverse, so that species identification of large samples is time-consuming and compels the involvement of taxonomic experts (Gotelli 2004). An established strategy to circumvent species-level identifications is taxonomic surrogacy, whereby specimens are assigned to higher taxonomic levels (e.g., family, order) for statistical analysis, under the mostly untested assumption that detection of experimental effects will not be altered. While taxonomic surrogacy simplifies the analysis of these complex data and is sometimes able to adequately capture the pattern of interest, it can also mask key interactions and lead to unacceptable losses of information about the system (Timms et al. 2013; Heino 2014). Moreover, the specific identity of predators also plays a role in the structuring of communities, but insights into their top-down and intraguild effects are mostly based on highly simplified food webs (Schmitz and Suttle 2001; Schneider and Brose 2013).

Ants represent a third of the animal biomass in terrestrial systems (Hölldobler and Wilson 1990) and are dominant components of arthropod communities, where they play multiple roles that lead to opposing effects on community structure. As generalist predators, ants can reduce herbivore populations and increase plant fitness but can also negatively affect other predators and lead to the disruption of trophic cascades (Rosumek et al. 2009; Sanders et al. 2011; but see Marín et al. 2014). In addition, some predatory ants are mutualists with honeydew-producing hemipterans such as aphids. This is a food-for-protection mutualism where ants tend hemipterans to feed on their honeydew and attack their predators in return (Buckley 1987). Despite the large body of experiments examining the effects of ants on terrestrial systems, most of them consider the effects of multiple ant species to be identical and manipulate the whole ant assemblage as a single entity for experimental purposes (but see Sanders and Van Veen 2011), hence experimental comparisons of the impact of single species against the whole ant assemblage are needed.

In the present study we use an organic citrus grove as a study system to disentangle the effect of a single versus multiple predators on a natural arthropod community where both intraguild prey and primary consumers are represented by multiple species. We wanted to tell apart the effect of *Lasius grandis* Forel from that of the rest of the crawling insect predators in the canopies, that is, ants and earwigs. *L. grandis* is a typical ant of citrus groves that has a dual role as a generalist predator and aphid tender (Pekas et al. 2011). Our grove hosts a diverse assemblage of ants and earwigs that crawl up to the canopies to forage, and excluding them from the canopies with a glue band usually has large effects on the arthropod community, likely stemming from a combination of foraging, mutualisms with aphids, and trait-mediated effects (Piñol et al. 2012; Mestre et al. 2014). In contrast, excluding birds by caging trees has at best modest effects (Piñol et al. 2010; Mestre et al. 2012, 2013a).

Given the diversity of crawling predators in the system, the effects of an unselective exclusion with a glue band are difficult to interpret (Piñol et al. 2009). Therefore, a method has been developed allowing differential exclusion of crawling predators by coupling a glue barrier with tubes (Romeu-Dalmau et al. 2010), which only *L. grandis* uses to bypass the barrier and reach the canopies, resulting in the exclusion of all other crawling insect species (Fig. 1a). Here we use this method as an additional exclusion treatment with two main objectives: (1) to test the effect of *L. grandis* against all crawling predators (control trees), and (2) to test the effect *L. grandis* against the full exclusion of crawling predators (glue band). We expected that: (1) would show the relative contribution of *L. grandis* to the total effects of crawling predators; and that (2) would be able to show a distinctive impact of *L. grandis* on community structure, due to predation causing top-down effects and aphid tending reducing densities of aphid predators. An important element of this study is the use of species-level data for assemblage analysis of several arthropod groups, providing a high degree of information to resolve effects on community structure.

Materials and methods

Study site

The grove is located in La Selva del Camp (Catalonia, north-east Spain; 41°13'07"N, 1°8'35"E). The area has a Mediterranean climate, where spring and autumn are rainy, and winters and summers are dry. The grove has ca. 300 clementine trees (*Citrus clementina* var. *clemenules*) grafted on to the hybrid rootstock Carrizo citrange [*Poncirus trifoliata* (L.) Raf. × *Citrus sinensis* (L.) Osb.]. The

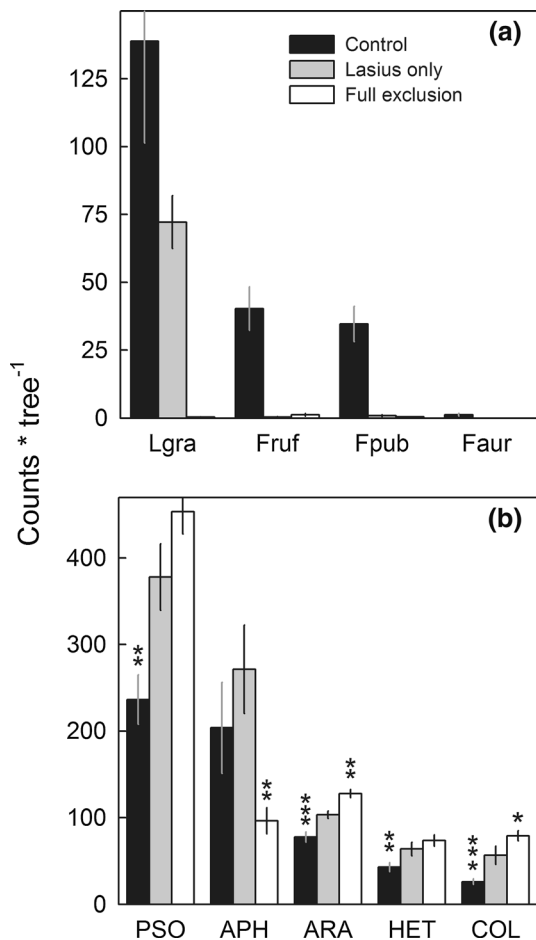


Fig. 1 Treatment effects on the densities of **a** crawling predators and **b** the main arthropod groups (mean \pm SE). The experiment modified the assemblage species composition of crawling predators (pseudo- $F_{2,23} = 11.32$, $P = 0.0001$) and the densities of all groups ($\chi^2_{2,23} > 77.42$, $P < 0.006$). **a** The most common ant and earwig species are shown but no univariate tests were made except on *Lasius grandis* (*Lgra*) densities, which were not significantly different between control and *Lasius*-only trees. **b** Asterisks show significant orthogonal post hoc contrasts focused on the *Lasius*-only treatment: control vs. *Lasius* only above black bars, full exclusion vs. *Lasius* only above white bars (** $P < 0.01$, *** $P < 0.001$). *Fruf* *Formica rufibarbis*, *Fpub* *Forficula pubescens*, *Faur* *Forficula auricularia*, *PSO* Psocoptera, *APH* Aphididae, *ARA* Araneae, *HET* Heteroptera, *COL* Coleoptera

trees are watered during dry periods and weeds are mowed regularly. Since 2005 the grove is fully organic, thus free of pesticide use.

Experimental design

We selected experimental trees at random from an area with 69 trees and we randomly assigned them to three treatments: full exclusion ($n = 9$), *Lasius* only ($n = 8$) and control ($n = 9$; see Online Resource 1). We applied a glue band on the trunk of full-exclusion trees to prevent ants and

earwigs from climbing up to the canopies. Some spider species are able to use the trunk to reach the canopies, but the assemblages of ground-dwelling and canopy-dwelling spiders are distinct (Mestre et al. 2013a). Silk-based aerial dispersal prevails in canopy-dwelling spiders, as evidenced by the fact that their densities increase in the trees where ants and earwigs have been excluded by a glue band (Mestre et al. 2013b). The sticky band was a sheet of plastic film sprayed with a polybutene-based glue (Rata Stop) and tightly attached over foam padding encircling the trunk. *Lasius*-only trees had the same glue band with an additional two translucent plastic tubes (Neoplast Ultravinil) of either 3- or 5-mm internal diameter underneath the padding cylinder. These tubes allowed *L. grandis* but not other ants or earwigs to access the canopies. In this system, 3-mm tubes exclude *Formica* ants, *Camponotus* ants and earwigs due to their large size (Romeu-Dalmau et al. 2010). In the present experiment, we initially had two sets of trees with tubes, of either 3- or 5-mm internal diameter ($n = 9$ each). We expected that at least *Formica* ants would reach the canopies with 5-mm tubes, resulting in an additional predator-manipulation treatment. However, we had to discard this idea when we realized that either *L. grandis* monopolized tubes of both diameters as gateways to the canopies or tubed trees had no or almost no ants over the whole sampling period (see Table 1 in Online Resource 2 showing ant densities by day and by night in an additional survey: *L. grandis* is as abundant in the tree canopies by night as it is by day). Thus, we retained the *Lasius*-only treatment by using the trees with tubes with the highest *L. grandis* densities (52–137 individuals), thereby discarding ten trees that had 0, 1, 4 or 11 individuals so that *Lasius*-only trees had more than four times as many individuals as the discarded trees (see Table 2 in Online Resource 2 showing the densities of each ant species in all trees and the choice of trees for the analyses, along with two alternative choices. Table 3 shows that the outcomes of analyzing the data based on these three alternatives are essentially the same). In full-exclusion and *Lasius*-only trees, ants may reach the canopies using tall weeds as bridges or dead insects as stepping stones over the glue band, so we inspected the trees weekly, and we cut weeds and replaced plastic films if needed. Control trees had natural densities of ants and earwigs. We established the treatments in January and sampled the trees once a month from February to December 2010.

Sampling methodology and taxonomic identifications

We sampled all arthropods on a monthly basis using beating trays with three blows in opposite directions on the canopies, and we captured them with entomological aspirators to preserve them in 70 % ethanol. We identified individuals to species with several taxonomic keys (see Online

Resource 3). If we were only able to identify immature individuals to genus (or family), we assigned them to the same species as adults if that was the only species of that genus in the grove.

Statistical analyses

The response variable was the cumulative densities of individuals of each species in each tree from February to December. We first checked the effectiveness of the treatments in modifying crawling insect densities with permutational multivariate ANOVA (PERMANOVA) on square-root-transformed species densities, with the Bray-Curtis index of similarity and treatment (full exclusion, *Lasius* only, control) as a fixed factor. PERMANOVA is a non-parametric analogue to multivariate ANOVA that circumvents the requirement of a multivariate normal distribution, which data on assemblage species composition rarely meet (Anderson 2001). We also conducted post hoc analyses to test for differences between treatments, with two orthogonal contrasts: control vs. full exclusion and *Lasius* only vs. full exclusion. We conducted a further analysis to test specifically for differences in *L. grandis* densities between control and *Lasius* only using a univariate generalized linear model (GLM) with the Poisson distribution corrected for overdispersion.

Second, to have a general idea of the experimental effects on canopy arthropods, we conducted a univariate analysis on the densities of each of the considered groups (psocopterans, aphids, spiders, true bugs, beetles) with treatment as a fixed factor using GLM with the Poisson distribution. We conducted a post hoc test for each significant GLM to examine differences between treatments with two planned orthogonal contrasts: *Lasius* only vs. full exclusion and *Lasius* only vs. control. Our purpose with these contrasts was to compare the effects of the *Lasius*-only treatment against each of the two other treatments, according to our goal of discerning the impact of *L. grandis* on the arthropod community.

Next, we tested for differences between treatments in the species composition of each arthropod group with PERMANOVAs on square-root-transformed species densities, using the Bray-Curtis index of similarity and treatment as a fixed factor. We also conducted a post hoc test for each significant PERMANOVA with orthogonal contrasts focused on the *Lasius*-only treatment, as just described.

When the PERMANOVA on the assemblage composition of a given arthropod group was significant, we conducted univariate analyses on the most common species in that group (mean >4 individuals per sample in at least one treatment, 29 species in total) with treatment as a fixed factor using GLM with the Poisson distribution. These univariate analyses enabled us to detect which species were causing the differences in assemblage composition between

treatments. As before, to analyze treatment differences in each significant GLM we conducted a post hoc test with orthogonal contrasts focused on the *Lasius*-only treatment.

Moreover, we assigned each of these common species (except aphids) to a trophic category, either predator or primary consumer, and performed a GLM on the densities of each trophic group, again with treatment as a fixed factor and the Poisson distribution. Unfortunately, a classification based on trophic categories of the more than 190 species occurring in our system was not possible due to the knowledge gaps in the literature about the feeding habits of coleopteran and heteropteran species (Online Resource 3).

To adjust the level of significance in our analyses we used the step-up false discovery rate (García 2004). We considered a ‘family’ of tests each of the following sets of analyses: the set of GLM on the total densities of each group, the set of PERMANOVA on the species composition of each group, each of the sets of GLM on species densities after a significant PERMANOVA, and also the sets of GLM on trophic group densities. Therefore, we adjusted the significance level in each of these families. Finally, we controlled the significance level in the post hoc tests. We used R version 3.0.2 (R Core Team 2013) for our analyses, with the package *vegan* for PERMANOVA (Oksanen et al. 2013).

Results

Exclusion of crawling predators

The treatments strongly modified densities of crawling predators (PERMANOVA, pseudo- $F_{2,23} = 11.32$, $P = 0.0001$; Fig. 1a), with the glue band being highly effective in excluding ants and earwigs from the canopies (control vs. full exclusion, $P = 0.0001$), and the tubes allowing *Lasius grandis* but no other crawling predators to colonize the canopies (*Lasius* only vs. full exclusion, $P = 0.0001$). There were almost half as many individuals in *Lasius*-only trees as in control trees, but these differences were not significant (GLM: $\chi^2_{1,15} = 180.11$, $P = 0.067$).

In control trees, we sampled 1641 ant individuals belonging to eight species. *L. grandis* (76.17 % of the individuals) and *Formica rufibarbis* Fabricius (22.12 %) were the most abundant ants. Other species were *Formica subrufa* Roger (0.73 %), *Tapinoma nigerrimum* (Nylander) (0.49 %) and *Plagiolepis pygmaea* (Latreille) (0.24 %). All these species, except *P. pygmaea*, are known aphid tenders. There were also a few individuals of *Camponotus* ants: *Camponotus aethiops* (Latreille), *Camponotus sylvaticus* (Olivier) and *Camponotus foreli* Emery. We captured 323 earwigs in control canopies, 96.6 % of them were *Forficula pubescens* Gené, and the rest were *Forficula auricularia* Linnaeus.

Densities of analyzed arthropod groups

Aside from ants and earwigs, we captured 22,917 arthropod individuals with beating trays. The most abundant groups were psocopterans (40.29 % of all individuals), aphids (21.27 %), spiders (11.69 %), true bugs (6.82 %), beetles (6.12 %), hymenopterans excluding Formicidae (3.78 %) and dipterans (2.78 %). We did not consider hymenopterans and dipterans in our analyses because beating trays were not a suitable method with which to sample them due to their high flying capabilities.

The experiment greatly influenced the densities of all five studied groups (psocopterans, aphids, spiders, true bugs, beetles; $\chi^2_{2,23} > 77.42$, $P < 0.006$). Psocopterans, true bugs, spiders and beetles had higher densities in *Lasius*-only than in control trees ($P < 0.027$; Fig. 1b). Additionally, densities of spiders and beetles were lower in *Lasius*-only than in full-exclusion trees ($P < 0.042$), consistent with a negative effect of *L. grandis* on arthropod densities. In contrast to the other groups, aphid densities stayed the same between control and *Lasius*-only trees ($P = 0.29$), and became lower in full-exclusion than in *Lasius*-only trees ($P = 0.0078$), suggesting a positive effect of the tending behavior of *L. grandis* on aphid densities.

Assemblage species composition and species densities

The experimental treatments modified the species composition of the five analyzed groups (pseudo- $F_{2,23} > 3.91$, $P < 0.015$), with *Lasius*-only trees hosting different species assemblages than full-exclusion and control trees ($P < 0.017$). Exceptions were aphids and beetles, with equal species assemblages between *Lasius*-only and control trees ($P > 0.076$; see also Table 3 in Online Resource 2).

Psocopterans

The five most common psocopteran species were *Ectopso-cus briggsi* McLachlan (Ectopsocidae; 47.6 % of all individuals) and *Trichopsocus clarus* Banks (Trichopsocidae; 25.8 %), followed by immature Ectopsocidae (17.9 %) and immature Trichopsocidae (6.2 %), regarded as species for the purpose of our analyses, and finally *Lachesilla pedicularia* (Linnaeus) (Lachesillidae, 1.1 %). Ant presence affected all groups except immature Trichopsocidae ($\chi^2_{2,23} > 18.7$, $P < 0.033$; Fig. 2a), these effects being entirely caused by lower densities in control than in *Lasius*-only trees ($P < 0.030$).

Aphids

The most abundant aphids were *Aphis* spp. nymphs (55.3 %), which we could not assign to any species,

followed by adults of *Aphis spiraeicola* Patch (27.9 %), *Aphis gossypii* Glover (15.4 %) and *Toxoptera aurantii* (Boyer de Fonscolombe) (1.2 %). Given the impossibility of telling apart *Aphis* spp. nymphs, we decided to perform the univariate analyses at the genus level. The analyses showed that the experiment only affected the genus *Aphis* ($\chi^2_{2,23} = 883.28$, $P = 0.0084$; Fig. 2b), with densities in *Lasius*-only trees being three times as high as in full-exclusion trees ($P = 0.018$) but equal to those in control trees ($P = 0.49$).

Spiders

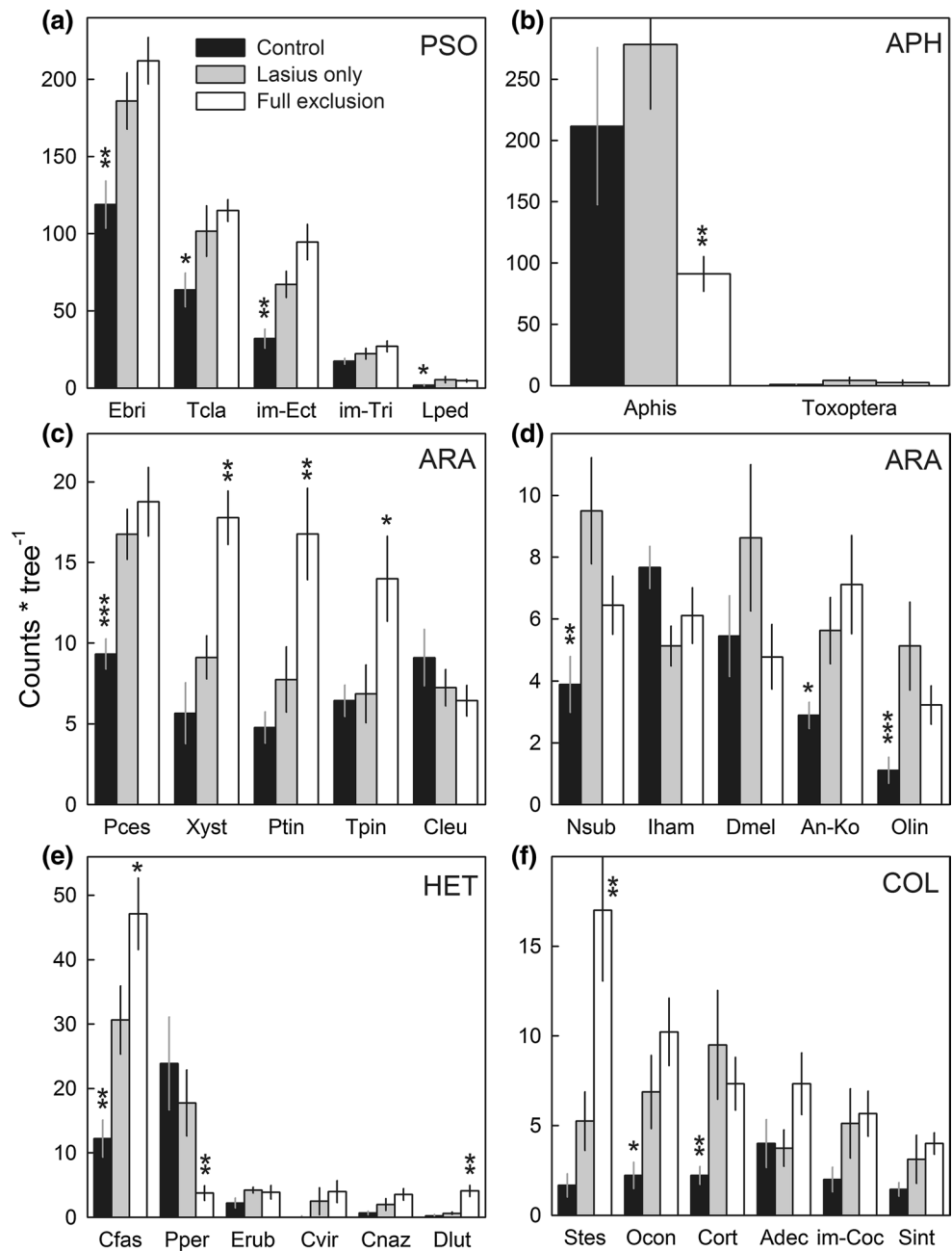
There were ten abundant spider species in the canopies, namely *Philodromus cespitum* (Walckenaer) (Philodromidae; 14.5 %), *Xysticus* sp. (Thomisidae; 10.6 %), *Platnickina tinctoria* (Walckenaer) (Theridiidae; 9.6 %), *Theridion pinastri* (C. L. Koch) (Theridiidae; 8.9 %), *Clubiona leucaspis* Simon (Clubionidae; 7.4 %), *Neoscona subfusca* (C. L. Koch) (Araneidae; 6.3 %), *Icius hamatus* (C. L. Koch) (Salticidae; 6.2 %), *Dipoena melanogaster* (C. L. Koch) (Theridiidae; 6 %), *Anelosimus-Kochiura* (Theridiidae; 5 %) and *Oxyopes lineatus* Latreille (Oxyopidae; 3 %). All *Xysticus* sp. individuals we collected were juveniles so we could not identify them to species. There were several juveniles compatible with both *Anelosimus vittatus* (C. L. Koch) and *Kochiura aulica* (C. L. Koch) (both Theridiidae), which are identical in appearance, so we pooled them into one group and treated them as a single species.

The treatments changed the densities of all spider species except *C. leucaspis*, *D. melanogaster* and *I. hamatus* ($\chi^2_{2,23} > 32.86$, $P < 0.0055$; Fig. 2c, d). In *P. cespitum*, *N. subfusca*, *Anelosimus-Kochiura* and *O. lineatus* the change was due to higher densities in *Lasius*-only trees than in control trees ($P < 0.0037$). Contrastingly, densities of *Xysticus* sp., *P. tinctoria* and *T. pinastri* in full-exclusion trees were twice as high as in *Lasius*-only trees ($P < 0.0055$) and hence not changed by any ant (and earwig) species other than *L. grandis*.

True bugs

The six most common species were *Cardiastethus fasciventris* (Garbiglietti) (Anthocoridae; 51.1 %), *Pilophorus perplexus* (Douglas and Scott) (Miridae; 25.7 %), *Empicoris rubromaculatus* (Blackburn) (Reduviidae; 5.8 %), *Campyloneura virgula* (Herrich-Schaeffer) (Miridae; 3.7 %), *Cardiastethus nazarenus* Reuter (Anthocoridae; 3.5 %) and *Deraeocoris lutescens* (Schilling) (Miridae; 2.9 %). *C. fasciventris*, *P. perplexus* and *D. lutescens* were affected by the treatments ($\chi^2_{2,23} > 47.60$, $P < 0.0013$; Fig. 2e). *C. fasciventris* and *D. lutescens* densities were higher in full-exclusion than in *Lasius*-only trees

Fig. 2 Treatment effects on species densities within each group (a–f; mean \pm SE). The experiment changed the assemblage species composition of every group (pseudo- $F_{2,23} > 3.91$, $P < 0.015$). As in Fig. 1, asterisks show significant orthogonal post hoc contrasts focused on the *Lasius*-only treatment ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$). *Ebri* *Ectoposocus briggsi*, *Tcla* *Trichopsocus clarus*, *im-Ect* immature Ectoposocidae, *im-Tri* immature Trichopsocidae, *Lped* *Lachesilla pedicularia*, *Pces* *Philodromus cespitum*, *Xyst* *Xysticus* sp., *Ptin* *Platnickina tincta*, *Tpin* *Theridion pinastri*, *Cleu* *Clubiona leucaspis*, *Nsub* *Neoscona subfusca*, *Iham* *Icius hamatus*, *Dmel* *Dipoena melanogaster*, *An-Ko* *Anelosimus-Kochiura*, *Oxy* *Oxyopes lineatus*, *Cfas* *Cardiastethus fasciventris*, *Pper* *Pilophorus perplexus*, *Erub* *Empicoris rubromaculatus*, *Cvir* *Campyloneura virgula*, *Cnaz* *Cardiastethus nazarenus*, *Dlut* *Deraeocoris lutescens*, *Stes* *Stilbus testaceus*, *Ocon* *Oenopia conglobata*, *Cort* *Corticaria* sp., *Adec* *Adalia decempunctata*, *im-Coc* immature Coccinellidae, *Sint* *Scymnus interruptus*



($P < 0.034$). *P. perplexus* densities showed the opposite response, with more than a fourfold increase from the full-exclusion to the *Lasius*-only trees ($P = 0.026$). The experiment changed *C. nazarenus* densities ($\chi^2_{2,23} = 46.61$, $P = 0.011$), but *Lasius*-only trees were no different than the others ($P > 0.20$).

Beetles

Stilbus testaceus (Panzer) (Phalacridae; 15.5 %), *Oenopia conglobata* (Linnaeus) (Coccinellidae; 12.3 %), *Corticaria* sp. (Latridiidae; 12 %), *Adalia decempunctata*

(Linnaeus) (Coccinellidae; 9.7 %), *Scymnus interruptus* (Goeze) (Coccinellidae; 8.1 %) and immature coccinellids (5.5 %) were the six most common beetle groups. We could not further classify individuals of *Corticaria* sp. and of immature coccinellids, but we treated them as species in the analyses. The experiment modified the densities of *O. conglobata*, *Corticaria* sp. and *S. testaceus* ($\chi^2_{2,23} > 44.38$, $P < 0.0023$; Fig. 2f). Densities of the first two species were more than three times as high in *Lasius*-only as in control trees ($P < 0.027$). By contrast, *S. testaceus* densities were three times as high in full-exclusion as in *Lasius*-only trees ($P = 0.0033$).

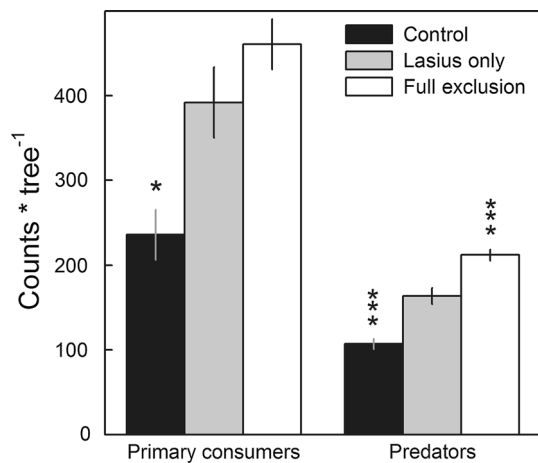


Fig. 3 Treatment effects on trophic group densities: **a** primary consumers except aphids, **b** predators (mean \pm SE). Both trophic groups were affected by the experiment ($\chi^2_{2,23} > 390.12$, $P < 0.026$). As in Fig. 1, asterisks show significant orthogonal post hoc contrasts focused on the *Lasius*-only treatment (* $P < 0.05$, *** $P < 0.001$)

Trophic categories

The experiment also affected the densities of primary consumers and predators among the most common species in our system ($\chi^2_{2,23} > 390.12$, $P < 0.026$; Fig. 3). Primary consumers comprised all psocopteran species and the mycophagous beetle *Corticaria* sp. (Majka et al. 2009), and predators comprised the rest of the beetle species, and all spider and heteropteran species. There were fewer primary consumers and predators in control than in *Lasius*-only trees ($P < 0.014$). Predator densities increased from *Lasius*-only to full-exclusion trees ($P < 0.0001$). Aphids were analyzed separately from the rest of primary consumers (see above).

Discussion

Our results show a strong impact of ants on the arthropod community, and a community-wide influence of *Lasius grandis* on assemblage species composition and on species densities. The sole presence of *L. grandis* exerted a marked effect on assemblage species composition in all arthropod groups, and on species densities in all groups except psocopterans, whose densities only changed in the control treatment. In general, *L. grandis* exclusion led to a two- to threefold increase in population densities of species belonging to several groups (Fig. 2). This differential exclusion experiment uncovers the strong and diverse effects of a single ant species on an arboreal arthropod community.

Effects of ant exclusion on assemblage species composition

After our multivariate analyses revealed that the experiment caused strong shifts in assemblage species composition in all arthropod groups, we performed univariate analyses on the densities of the most common species in each group (29 in total) and found 18 of them (60 %) to be modified by the experiment. Remarkably, treatment effects on species densities never overlapped, since they were caused by *Lasius*-only trees differing from either control trees or full-exclusion trees, but not from both (the sole exception was the true bug *C. fasciventris*). Thus, we could distinguish two groups of species: those affected by the whole crawling predator assemblage (control vs. *Lasius* only) and those affected by *L. grandis* alone (*Lasius* only vs. full exclusion; Fig. 1).

Effect of the whole crawling predator assemblage

The ten species affected by the treatment comprised four psocopteran, four spider and two beetle species that had higher densities in *Lasius*-only than in control trees and no density changes between *Lasius*-only and full-exclusion trees. Hence, the presence of all ants and earwigs was negatively impacting species densities, whereas *L. grandis* alone lacked detectable effects. These differences between treatments can be imputed either to a higher number of ants and earwigs on control trees or to the diversity of ant and earwig species on them.

Effect of the ant *L. grandis*

The presence of *L. grandis* patrolling in the canopies of the trees with glue and tubes had a determining influence on assemblage species composition and on the densities of eight important arthropod species. This is emphasized by the fact that densities of these species did not vary between control and *Lasius*-only trees, which indicates that the combined impact of all ant species plus earwigs was negligible compared to the impact of *L. grandis* alone. Indeed, *L. grandis* densities in *Lasius*-only trees were enough to affect arthropod populations. In principle, these densities were almost half as low as in control trees, but the difference between treatments was not significant (Fig. 1a). Densities of three spiders, two true bugs and one beetle species were higher in full-exclusion than in *Lasius*-only trees, showing that the influence of *L. grandis* spread across disparate arthropod groups. As a single exception, densities of the true bug *C. fasciventris* were also higher in *Lasius*-only than in control trees, thus being possibly affected by predators other than *L. grandis*.

Conversely, densities of *Aphis* spp. and the true bug *P. perplexus* were boosted by the *Lasius*-only treatment compared to full exclusion. *P. perplexus* is a myrmecomorphic aphid predator (McIver and Stonedahl 1993) that in previous studies in the same grove was also found to prefer unmanipulated trees with ants, where it was spared from ant harassment (Piñol et al. 2009, 2012). Now, our differential exclusion experiment proves this association of *P. perplexus* with ants to be species-specific, since it holds true for *L. grandis* alone. Accordingly, our results also show that *L. grandis* was the only ant species whose aphid-tending behavior had a detectable positive effect on aphid densities, these being three times as high in *Lasius*-only as in full-exclusion trees (Fig. 2b). Previously only a glue band treatment was used (Piñol et al. 2009, 2012) and results on aphid densities were unclear, allegedly because the positive effect of ant tending was countered by the negative effect earwig predation, especially at the onset of the aphid season (Romeu-Dalmau et al. 2012). Our differential exclusion of *L. grandis* against the whole ant and earwig assemblage provides quantitative support for this assumption.

Diversity of within-group responses to predator presence

We tracked experimental effects down to species instead of remaining at the group level. These fine-scale analyses revealed that closely related species had contrasting responses to a given experimental treatment. This was the case of the spider and beetle assemblages, which contained species that were unaffected by *L. grandis* but that responded to the presence of the whole crawling predator assemblage, and vice versa (Fig. 2c, d, f). Therefore, group-level density changes in response to treatment (Fig. 1b) failed to reflect complex within-group ecological responses by masking species-specific effects. This was particularly striking in true bugs, where the only group-level change was an increase in densities from the control to the *Lasius*-only trees, whereas the densities of some species in this group changed dramatically between the *Lasius*-only and the full-exclusion trees (Fig. 2e). Researchers dealing with hyperdiverse communities are confronted with resource and time constraints that hinder species-level identifications, so they sidestep this handicap by using higher-taxa surrogates to analyze data. However, our experimental results show that responses at higher levels of the taxonomic hierarchy were a misleading summary of species-level responses, and we thus positively caution against overgeneralizing the taxonomic surrogacy approach (Maurer 2000).

Differential top-down and intraguild effects of *L. grandis* and the whole crawling predator assemblage

The ant and earwig assemblage in control trees comprised ten species and was largely dominated by *L. grandis*, *F.*

rufibarbis, and the earwig *F. pubescens*. Densities of *L. grandis* workers were lower in *Lasius*-only trees than in control trees, but non-significantly so, and densities of eight common arthropod species changed markedly between *Lasius*-only trees and full-exclusion trees, but not between *Lasius*-only and control trees. This shows a distinctive and far-reaching effect of *L. grandis* on the arthropod community, which could be due to its foraging strategy. The strategy of *L. grandis* is based on mass recruitment, whereby a large number of individuals gather along a foraging trail (Beckers et al. 1992). By contrast, *F. rufibarbis*, while also a predator and aphid tender, has a foraging strategy based on workers collecting food in a solitary manner (Deffernez et al. 1990), and earwigs are solitary omnivorous insects that prey on aphids (Romeu-Dalmau et al. 2012).

As shown above, the effectiveness of *L. grandis* in guarding and defending *Aphis* spp. from predators led to a threefold increase in their densities in *Lasius*-only trees compared to full exclusion. Consistent with this outcome, the six arthropod species negatively affected by *L. grandis* were generalist predators known to feed on aphids, among which spiders [*Xysticus* sp., *P. tinctoria*, *T. pinastri* (Marc and Canard 1997; Birkhofer et al. 2008)], true bugs [*D. lutescens*, *C. fasciventris* (Fauvel 1999)], and the beetle *S. testaceus* (Chu 1949). Surprisingly, *L. grandis* did not influence densities of coccinellid beetles (*A. decempunctata*, *O. conglobata*, *S. interruptus*), which are specialized aphid predators (Hagen 1962), though *L. grandis* had an overall negative effect on predator densities (Fig. 3). Thus, our results show a prevalence of intraguild interactions between *L. grandis* and a suite of other arthropod predators that may be in part mediated by the food-for-protection mutualism with aphids (Buckley 1987; Pekas et al. 2011; Moreira et al. 2012). In fact, the increase in *Aphis* spp. densities was the only detected (positive) top-down effect of *L. grandis*. Strikingly, though *L. grandis* is a generalist predator, its removal from the canopies did not result in a boost in densities of primary consumers, which comprised basically psocopterans. Because in the canopies of our system psocopterans are the most important prey in terms of densities (Piñol et al. 2012; Mestre et al. 2013c), it is plausible to attribute the lack of detected top-down effects of *L. grandis* on psocopterans to the fact that *L. grandis* predation on these was compensated for by the *Lasius*-induced reduction in densities of generalist predators.

In contrast to the presence of *L. grandis* alone, the presence of all ant and earwig species in the canopies exerted negative top-down effects on the densities of primary consumers, as well as a negative effect on the densities of predators (Fig. 3). Experimental manipulations of overall ant densities in different systems often document a strong influence of foraging ant workers on primary consumers and predators in the arthropod community (Sanders and

Platner 2007; Piñol et al. 2012; Mestre et al. 2013b). Nevertheless, work attempting to disentangle the ecological impacts of different ant groups is wanting. In an original experiment, Moya-Laraño and Wise (2007) excluded ants based on body size and quantified their impacts on ground-dwelling spiders, showing that large ants (primarily *Camponotus* spp.) accounted for the observed effects of both large and small ants. To our knowledge, ours is the first study that experimentally compares the impact of one ant species (*L. grandis*) against the whole ant assemblage (plus earwigs). Our main finding was that exclusion of ants and earwigs (control vs. *Lasius* only) reduced top-down control on primary consumers but increased densities of some generalist predators, and that further removal of *L. grandis* (*Lasius* only vs. full exclusion) induced a rise in densities of additional generalist predator species without further dampening top-down control (densities of primary consumers did not increase; Fig. 3). Though the (non-significant) lower densities of *L. grandis* in *Lasius*-only than in control trees may have also contributed to the observed outcomes, our results reveal, in general, that *L. grandis* has widespread intraguild effects on generalist predators, but that top-down control of herbivores necessitates involvement of the whole ant and earwig assemblage.

Because experimentally isolating the effects of different predators is fraught with logistic difficulties, researchers assume the predators of a particular system to have similar effects, even when they belong to disparate taxonomic groups. However, solid evidence to the contrary is building up for different predator groups [ants vs. birds (Mooney 2006, 2007; Piñol et al. 2010); birds vs. bats (Maas et al. 2013; Karp and Daily 2014)]. Our study demonstrates that even within a seemingly homogeneous group of predators (ten species of ants and earwigs), the influence of a single species (*L. grandis*) on community structure can turn out to be very different from the combined impact of all predators.

Conclusion

Our differential exclusion of *L. grandis* against the rest of ants and earwigs allowed us to detect a diverse array of community-wide intraguild effects by a single predator that stand in stark contrast to the intraguild and top-down effects exerted by the whole crawling predator assemblage. By analyzing the effects on species assemblages of several arthropod groups, we demonstrate the distinctive and widespread influence of a single ant species on an arthropod community, and highlight the crucial importance of taking into account diversity at the species level in order to gain insights into the structuring of ecological communities.

Acknowledgments We are grateful to Núria Cañellas for assistance with the fieldwork and to the following colleagues for species identifications: Helena Pascual (Psocoptera), Nicolás Pérez (Aphididae), Jordi and Eva Ribes (Heteroptera), Josep Muñoz (Coleoptera).

Author contribution statement J. P., J. A. B., and X. E. designed and performed the experiment. L. M. processed the data, performed statistical analyses and wrote the manuscript; other authors provided editorial advice.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Funding Two grants from the Ministerio de Ciencia e Innovación-Fondo Europeo de Desarrollo Regional (MICINN-FEDER; CGL2007-64080-C02-01/BOS, CGL2010-18182) funded this study.

References

- Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecol* 26:32–46
- Arim M, Marquet PA (2004) Intraguild predation: a widespread interaction related to species biology. *Ecol Lett* 7:557–564. doi:10.1111/j.1461-0248.2004.00613.x
- Beckers R, Deneubourg J-L, Goss S (1992) Trail laying behaviour during food recruitment in the ant *Lasius niger* (L.). *Insectes Soc* 39:59–72
- Birkhofer K, Gavish-Regev E, Endlweber K, Lubin YD, von Berg K, Wise DH, Scheu S (2008) Cursorial spiders retard initial aphid population growth at low densities in winter wheat. *Bull Entomol Res* 98:249–255. doi:10.1017/S0007485308006019
- Buckley RC (1987) Interactions involving plants, Homoptera, and ants. *Annu Rev Ecol Syst* 18:111–135
- Chu HF (1949) How to know the immature insects. Brown, Dubuque
- Davenport JM, Chalcraft DR (2012) Evaluating the effects of trophic complexity on a keystone predator by disassembling a partial intraguild predation food web. *J Anim Ecol* 81:242–250. doi:10.1111/j.1365-2656.2011.01906.x
- Deffernez L, Champagne P, Verhaeghe J-C, Josens G, Loreau M (1990) Analysis of the spatio-temporal niche of foraging grassland ants in the field. *Insectes Soc* 37:1–13
- Fauvel G (1999) Diversity of Heteroptera in agroecosystems: role of sustainability and bioindication. *Agric Ecosyst Environ* 74:275–303. doi:10.1016/S0167-8809(99)00039-0
- Finke DL, Denno RF (2005) Predator diversity and the functioning of ecosystems: the role of intraguild predation in dampening trophic cascades. *Ecol Lett* 8:1299–1306. doi:10.1111/j.1461-0248.2005.00832.x
- García LV (2004) Escaping the Bonferroni iron claw in ecological studies. *Oikos* 105:657–663
- Gotelli NJ (2004) A taxonomic wish-list for community ecology. *Philos Trans R Soc Lond B Biol Sci* 359:585–597. doi:10.1098/rstb.2003.1443
- Hagen KS (1962) Biology and ecology of predaceous Coccinellidae. *Annu Rev Entomol* 7:289–326
- Hairston NG, Smith FE, Slobodkin LB (1960) Community structure, population control, and competition. *Am Nat* 94:421–425
- Heino J (2014) Taxonomic surrogacy, numerical resolution and responses of stream macroinvertebrate communities to ecological gradients: are the inferences transferable among regions? *Ecol Ind* 36:186–194. doi:10.1016/j.ecolind.2013.07.022

- Hölldobler B, Wilson EO (1990) *The ants*. Harvard University Press, Cambridge
- Ives AR, Cardinale BJ, Snyder WE (2005) A synthesis of sub-disciplines: predator-prey interactions, and biodiversity and ecosystem functioning. *Ecol Lett* 8:102–116. doi:10.1111/j.1461-0248.2004.00698.x
- Karp DS, Daily GC (2014) Cascading effects of insectivorous birds and bats in tropical coffee plantations. *Ecology* 95:1065–1074. doi:10.1890/13-1012.1
- Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: a review and prospectus. *Can J Zool* 68:619–640
- Maas B, Clough Y, Tschamtké T (2013) Bats and birds increase crop yield in tropical agroforestry landscapes. *Ecol Lett* 16:1480–1487. doi:10.1111/ele.12194
- Majka CG, Langor D, Rucker WH (2009) Latridiidae (Coleoptera) of Atlantic Canada: new records, keys to identification, new synonyms, distribution, and zoogeography. *Can Entomol* 141:317–370. doi:10.4039/1109-050
- Marc P, Canard A (1997) Maintaining spider biodiversity in agroecosystems as a tool in pest control. *Agric Ecosyst Environ* 62:229–235. doi:10.1016/S0167-8809(96)01133-4
- Marín L, Jackson D, Perfecto I (2014) A positive association between ants and spiders and potential mechanisms driving the pattern. *Oikos*. doi:10.1111/oik.01913
- Maurer D (2000) The dark side of taxonomic sufficiency (TS). *Mar Pollut Bull* 40:98–101. doi:10.1016/S0025-326X(99)00235-0
- McIver JD, Stonedahl G (1993) Myrmecomorphy: morphological and behavioral mimicry of ants. *Annu Rev Entomol* 38:351–379
- Mestre L, Piñol J, Barrientos JA, Cama A, Espadaler X (2012) Effects of ant competition and bird predation on the spider assemblage of a citrus grove. *Basic Appl Ecol* 13:355–362. doi:10.1016/j.baae.2012.04.002
- Mestre L, García N, Barrientos JA, Espadaler X, Piñol J (2013a) Bird predation affects diurnal and nocturnal web-building spiders in a Mediterranean citrus grove. *Acta Oecol* 47:74–80. doi:10.1016/j.actao.2013.01.001
- Mestre L, Piñol J, Barrientos JA, Espadaler X (2013b) Ant exclusion in citrus over an 8-year period reveals a pervasive yet changing effect of ants on a Mediterranean spider assemblage. *Oecologia* 173:239–248. doi:10.1007/s00442-013-2594-y
- Mestre L, Piñol J, Barrientos JA, Espadaler X, Brewitt K, Werner C, Platner C (2013c) Trophic structure of the spider community of a Mediterranean citrus grove: a stable isotope analysis. *Basic Appl Ecol* 14:413–422. doi:10.1016/j.baae.2013.05.001
- Mestre L, Bucher R, Entling MH (2014) Trait-mediated effects between predators: ant chemical cues induce spider dispersal. *J Zool* 293:119–125. doi:10.1111/jzo.12127
- Mooney KA (2006) The disruption of an ant-aphid mutualism increases the effects of birds on pine herbivores. *Ecology* 87:1805–1815. doi:10.1890/0012-9658(2006)87[1805:TDOAA M]2.0.CO;2
- Mooney KA (2007) Tritrophic effects of birds and ants on a canopy food web, tree growth, and phytochemistry. *Ecology* 88:2005–2014. doi:10.1890/06-1095.1
- Moreira X, Mooney KA, Zas R, Sampedro L (2012) Bottom-up effects of host-plant species diversity and top-down effects of ants interactively increase plant performance. *Proc R Soc B Biol Sci* 279:4464–4472. doi:10.1098/rspb.2012.0893
- Moya-Laraño J, Wise DH (2007) Direct and indirect effects of ants on a forest-floor food web. *Ecology* 88:1454–1465. doi:10.1890/05-1474
- Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H (2013) *Vegan: community ecology package*. R package version 2.0.0-10. <http://CRAN.R-project.org/package=vegan>
- Pekas A, Tena A, Aguilar A, Garcia-Marí F (2011) Spatio-temporal patterns and interactions with honeydew-producing Hemiptera of ants in a Mediterranean citrus orchard. *Agric For Entomol* 13:89–97. doi:10.1111/j.1461-9563.2010.00501.x
- Piñol J, Espadaler X, Cañellas N, Pérez N (2009) Effects of the concurrent exclusion of ants and earwigs on aphid abundance in an organic citrus grove. *Biocontrol* 54:515–527. doi:10.1007/s10526-008-9203-8
- Piñol J, Espadaler X, Cañellas N, Martínez-Vilalta J, Barrientos JA, Sol D (2010) Ant versus bird exclusion effects on the arthropod assemblage of an organic citrus grove. *Ecol Entomol* 35:367–376. doi:10.1111/j.1365-2311.2010.01190.x
- Piñol J, Espadaler X, Cañellas N (2012) Eight years of ant-exclusion from citrus canopies: effects on the arthropod assemblage and on fruit yield. *Agric For Entomol* 14:49–57. doi:10.1111/j.1461-9563.2011.00542.x
- Polis GA, Myers CA, Holt RD (1989) The ecology and evolution of intraguild predation: potential competitors that eat each other. *Annu Rev Ecol Syst* 20:297–330
- R Development Core Team (2013) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna. <http://www.R-project.org/>
- Romeu-Dalmau C, Espadaler X, Piñol J (2010) A simple method to differentially exclude ants from tree canopies based on ant body size. *Methods Ecol Evol* 1:188–191. doi:10.1111/j.2041-210X.2010.00015.x
- Romeu-Dalmau C, Pinol J, Agustí N (2012) Detecting aphid predation by earwigs in organic citrus orchards using molecular markers. *Bull Entomol Res* 102:566–572. doi:10.1017/S0007485312000132
- Rosumek FB, Silveira FA, Neves FdS, Barbosa NPdU, Diniz L, Oki Y, Pezzini F, Fernandes GW, Cornelissen T (2009) Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. *Oecologia* 160:537–549. doi:10.1007/s00442-009-1309-x
- Sanders D, Platner C (2007) Intraguild interactions between spiders and ants and top-down control in a grassland food web. *Oecologia* 150:611–624. doi:10.1007/s00442-006-0538-5
- Sanders D, van Veen FJ (2011) Ecosystem engineering and predation: the multi-trophic impact of two ant species. *J Anim Ecol* 80:569–576. doi:10.1111/j.1365-2656.2010.01796.x
- Sanders D, Schaefer M, Platner C, Griffiths GJK (2011) Intraguild interactions among generalist predator functional groups drive impact on herbivore and decomposer prey. *Oikos* 120:418–426. doi:10.1111/j.1600-0706.2010.18924.x
- Schmitz OJ (2007) Predator diversity and trophic interactions. *Ecology* 88:2415–2426. doi:10.1890/06-0937.1
- Schmitz OJ, Suttle KB (2001) Effects of top predator species on direct and indirect interactions in a food web. *Ecology* 82:2072–2081. doi:10.1890/0012-9658(2001)082[2072:EOTPSO]2.0.CO;2
- Schmitz OJ, Hawlena D, Trussell GC (2010) Predator control of ecosystem nutrient dynamics. *Ecol Lett* 13:1199–1209. doi:10.1111/j.1461-0248.2010.01511.x
- Schneider FD, Brose U (2013) Beyond diversity: how nested predator effects control ecosystem functions. *J Anim Ecol* 82:64–71. doi:10.1111/1365-2656.12010
- Sih A, Englund G, Wooster D (1998) Emergent impacts of multiple predators on prey. *Trends Ecol Evol* 13:350–355. doi:10.1016/S0169-5347(98)01437-2
- Sitvarin MI, Rypstra AL (2014) The importance of intraguild predation in predicting emergent multiple predator effects. *Ecology* 95:2936–2945. doi:10.1890/13-2347.1
- Timms LL, Bowden JJ, Summerville KS, Buddle CM, Didham R, Basset Y (2013) Does species-level resolution matter? Taxonomic sufficiency in terrestrial arthropod biodiversity studies. *Insect Conserv Divers* 6:453–462. doi:10.1111/icad.12004

- Vance-Chalcraft HD, Rosenheim JA, Vonesh JR, Osenberg CW, Sih A (2007) The influence of intraguild predation on prey suppression and prey release: a meta-analysis. *Ecology* 88:2689–2696. doi:[10.1890/06-1869.1](https://doi.org/10.1890/06-1869.1)
- Werner EE, Peacor SD (2003) A review of trait-mediated indirect interactions in ecological communities. *Ecology* 84:1083–1100. doi:[10.1890/0012-9658\(2003\)084\[1083:AROTII\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2003)084[1083:AROTII]2.0.CO;2)