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Additional Information

Running head: ants and natural enemies

Title: Ants in citrus: impact on the abundance, species richness, diversity and community structure of predators and parasitoids

Authors: Altea Calabuig[a], Ferran Garcia-Marí[a], Apostolos Pekas[a]

[a]Instituto Agroforestal Mediterráneo (IAM), Universitat Politècnica de València, Camí de Vera s/n, 46022, València, Spain

Corresponding author:

Altea Calabuig Gomar, alteac@outlook.com Tel. +34651995119, Fax +34963877331

E-mail address: alteac@outlook.com

Abstract

Although ants act as plant biotic defences, in agricultural ecosystems they are often associated with outbreaks of honeydew-producing pests mainly due to the protection they offer to the plant feeders in exchange for honeydew. In such an interaction ants may alter the abundance, diversity and community structure of predators and parasitoids. In the present study, we conducted ant-exclusion experiments in three commercial citrus orchards, each one dominated by one ant species (*Pheidole pallidula, Lasius grandis* or *Linepithema humile*) during two consecutive years (2011 and 2012). We then compared the abundance, species richness, diversity and community structure of predators and parasitoids between the ant-allowed and ant-excluded treatments. A total of 176,000 arthropods belonging to 81 taxa were captured and identified. Regarding abundance, our results showed a species specific response between treatments. When examining functional groups, in the ant-allowed treatment, seven species of predators decreased and four increased in abundance, whereas four species of parasitoids decreased and 18 increased in abundance. The species richness (S) was significantly lower for predators and higher for parasitoids in the ant-allowed treatment. The Shannon diversity index (H) was not different between treatments in the case of predators, whereas in the case of parasitoids diversity was significantly higher in the ant-allowed treatment. Finally, the community structure of predators and parasitoids was not significantly different between treatments. These results suggest that ants in the citrus agroecosystem are not associated with a dramatic decrease in natural enemy abundance or biodiversity; on the contrary ants were associated with increased parasitoid species richness and diversity. Despite the fact that ants have no negative impact on the abundance and diversity of predators and parasitoids at the community level their impact on specific natural enemy species, mainly predators, may explain the highest pest densities associated with ants in citrus.

Keywords: biodiversity, biological control, ant exclusion, citrus agroecosystems, multitrophic interactions

1. Introduction

Ants are keystone species affecting directly and indirectly the ecosystem structure and functioning. Ants may act as soil tillers, seed dispersers, pollinators (Beattie, 1985), predators (Karhu, 1998; Olotu et al., 2013) and are involved in various mutualisms (Rosumek et al., 2009; Way, 1963). Especially mutualisms have been found to have broader effects on the wider arthropod community affecting eventually plant health (Eubanks and Finke, 2014; Kaplan and Eubanks, 2005). One of the best studied mutualisms involving ants is the relationship with honeydew producing hemipterans, in which ants use the honeydew excreted as an important carbohydrate source and, in turn, protect hemipterans from their natural enemies (Bartlett, 1961; Carroll and Janzen, 1973; Hölldobler and Wilson, 1990; Way, 1963). As a result, ant-tending may have wider community-level consequences by altering the abundance and distribution of predators and parasitoids (Styrsky and Eubanks, 2007; Tena et al., 2013). These interactions play an important role in natural and most crucially in agricultural ecosystems since biological pest control provided by predators and parasitoids, an important ecosystem service (Naylor and Ehrlich, 1997), may be negatively affected by ant activity (DeBach et al., 1951; Martínez-Ferrer et al., 2003)

The impact of ants on natural enemy abundance, diversity or community structure varies considerably depending on the natural enemy species as well as on the species of ants involved or the ecosystem where the study took place (Table 1 and references therein). Whereas several studies have demonstrated a negative impact of ants on the abundance of natural enemies (Eubanks, 2001; James et al., 1999; Kaplan and Eubanks, 2005; Piñol et al., 2012a) others find no effect (Chong et al., 2010; Gibb, 2003; Offenberg et al., 2005) or even find positive effects of ants on the community of natural enemies (Peng and Christian, 2013; Stewart-Jones et al., 2007). The same or even greater variability is reported at the species level; natural enemies, even species belonging to the same taxonomic order, may be affected differently by ants. For example, several studies have shown that ants have a negative impact on certain coccinellid species (Bartlett, 1961; Itioka and Inoue, 1996; Kaplan and Eubanks, 2002), but other coccinellid species are not affected (Flanders, 1958; Vanek and Potter, 2010) or even increase their densities under ant presence (Daane et al., 2007; Völkl and Vohland, 1996). Likewise, the activity of several parasitoid species is disrupted by ants (Bartlett, 1961; Martínez-Ferrer et al., 2003; Mgocheki and Addison, 2009) while others are able to parasitize ant-tended plant feeders (Barzman and Daane, 2001; Flanders, 1958; Völkl, 1994).

Several studies have examined the multitrophic interactions involving ants and natural enemies in citrus in different parts of the world (Dao et al., 2014; James et al., 1999; Piñol and Espadaler, 2010; Piñol et al., 2012a; Yoo et al., 2013) yet again there are no studies examining the impact of ants simultaneously on predator and parasitoid species. Most of the studies focus on predators from different taxa but little is known about the impact of ants on the diversity and abundance of parasitoids, which are often studied as a single group in the order Hymenoptera. Knowledge about the impact of ants on the abundance, diversity and community structure of natural enemies in the citrus agroecosystem will provide useful insights and can help us to clarify the role of ants in biological control.

Thus, our research focuses on determining the impact of ants on the abundance, diversity and community structure of predators and parasitoids in Mediterranean citrus orchards. We performed an ant-exclusion experiment during two consecutive years in three commercial citrus orchards each one with a different dominant ant species. The main objectives of the study were: (1) to know, describe and quantify the community of predators and parasitoids in the three citrus orchards (2) to test whether ants impact the abundance of predators and parasitoids and (3) to test whether ants impact the species richness, diversity and community structure of predators and parasitoids.

2. Materials and methods

2.1 Study sites

The study was conducted during two consecutive growing seasons, from April 2010 to November 2011, in three commercial citrus orchards located in an extensive citrus-growing area located 30 km south of Valencia, eastern Spain (39º 12' N, 0º 20' W; 39º 11' N, 0º 20' W and 39º 14' N, 0º 15' W). The climate is Mediterranean, with a rainy spring and autumn and a dry winter and summer. The orchards were flood irrigated and weeds were controlled by local application of herbicides (Glyphosate®, Bayer CropScience, Spain). Two orchards were of sweet orange *Citrus sinensis* (L.) Osbeck (cv. Navelina) and one of a mixture of two species, sweet orange *C. sinensis* (cv. Navelina) and Clementine mandarin *Citrus reticulata* Blanco (Cv. Clementina Fina). In all orchards trees were more than 10 years old. No insecticides were sprayed in the previous five years or during the two-year experimental period. In each orchard, a behaviourally dominant ant species was present. From now on we will refer to the orchards according to the acronym of the predominant ant species present. Thus, in the orchard PP the predominant ant species was *Pheidole pallidula* (Nylander), in the orchard LG the predominant ant species was *Lasius grandis* Forel and in the orchard LH *Linepithema humile* (Mayr) was the only ant species present and foraging on the tree canopies (for details see Calabuig et al., 2013).

2.2 Experimental design

At each orchard, the experimental design was a randomized block with four replicates (blocks) of two adjacent treatments (plots): ant-allowed and ant-excluded trees. Each plot contained 16 trees (four rows by four trees per row). Ants were excluded in the 16 trees of the ant-excluded plots and left unaffected in the 16 trees of the ant-allowed plots. Only the four central trees of each plot were used for the samplings. With that method we ensure that arthropods captured came from the trees of the same plot and corresponding treatment. Ant-exclusion began in April 2011 and was maintained until November 2012 (19 months). During the first season (2011), ant exclusion was achieved by painting a 25-cm wide band of insecticidal paint in a micro-encapsulated formulation (Inesfly FITO© (chlorpyrifos 3%)), Industrias Químicas Inesba S.L., Paiporta, Spain) on the trunk. To ensure that no ants reached the tree canopies, ant-excluded trees were inspected every month and the band was repainted if ants were observed crossing the band. Due to the fact that we observed ants crossing the painted bands in some of the trees during the first growing season we changed the ant exclusion method during the subsequent season. Thus, during 2012, ant exclusion was conducted by applying Tangle-trap® (Tanglefoot, Biagro, Valencia, Spain) sticky barrier on the tree trunks. Sticky barriers were inspected every month and, if necessary, the Tanglefoot was renewed; in any case, Tanglefoot was renewed routinely every two months. Trees were pruned periodically and ground vegetation was trimmed to prevent alternative ways for ants to reach the canopies.

2.3 Arthropod sampling and classification

Arthropods on the tree canopies were sampled with yellow sticky traps and by using an aspiration vacuum device. In each plot, one yellow sticky trap (Bug-scan, Biobest®), 100 mm x 250 mm, was placed at 1.60 m high in the middle of the plot by hanging it on a twig. Aspiration samples were taken from the four central trees of each plot using a modified vacuum sampler (Komatsu Zenoah Co. HBZ2601) consisting on a reversed leaf-blower with a mesh bag to retain the sample. The vacuum sampler was applied on the canopies during one minute on each one of the four central trees of the plot. The sticky traps were replaced monthly, from April 2011 to November 2012 whereas aspiration samples were taken monthly from April to August and bimonthly from September to December in 2011 and 2012. Samples were transferred to the laboratory and maintained in a freezer until their identification.

All natural enemies captured were counted and identified. Most of them were identified to species or morphospecies level, while a few natural enemies were identified to genera or families. The use of morphospecies is a useful tool for studies that require taxonomic identifications of a great number of invertebrates without compromising scientific accuracy (Oliver and Beattie, 1996).

2.4 Predator and parasitoid diversity

The natural enemy diversity was measured in each sampling date by calculating the species richness *S* (number of species or morphospecies) and the Shannon diversity index *H′* (Shannon and Weaver, 1949):

$$H' = -\sum_{i=1}^{i=S} p_i \cdot log_e\, p_i$$

where p_i is the proportion of individuals of each species (up to a total of *S* species) in each sample. In the calculation of species richness and diversity we included the natural enemies identified to species or morphospecies level. Hyperparasitoid species and species belonging to the fourth trophic level were not included in these analyses.

2.5 Statistical analysis

All analysis were performed using all the captures of each month with both traps and aspiration samples (i.e. May, June, July, August, October and December for 2011 and April, May, June, July, August, September and November in 2012).

To compare the abundance of specific natural enemies in ant-allowed and ant-excluded treatments we included only those species with more than 40 individuals captured in total during the two seasons of the study in the three orchards. We applied repeated measures ANOVA on the abundance of every species, either at each orchard or globally, i.e. considering the three orchards together. Treatment (ant-excluded versus ant-allowed) was the fixed factor and orchard (in the global analysis), bloc (nested into orchard) and sampling date were random factors. Data were log-transformed in order to meet normality assumptions. All ANOVAS were conducted using Statgraphics 5.1 software (Statgraphics, 1994).

Repeated measures analysis of variance (ANOVA) were applied to compare the species richness (S) and the Shannon diversity index (H') in ant-allowed and ant-excluded treatments in each orchard and globally. Treatment (ant-excluded versus ant-allowed) was the fixed factor and orchard (in the global analysis), bloc (nested into orchard) and sampling date were random factors. For the community structure analysis we included all natural enemies identified to species or morphospecies. To compare the community structure of natural enemies in ant-allowed and ant-excluded treatments, permutational multivariate analyses of variance (PERMANOVA) were applied to predators and parasitoids abundances using the *adonis* function in the vegan package (Anderson, 2001; Oksanen et al., 2009) in R (R Development Core Team, 2014). A separate PERMANOVA was conducted in each orchard for each year. Distance matrices for use in PERMANOVA were constructed using the Bray-Curtis index, and P-values were generated using F-tests based on sequential sums of squares from 99999 permutations of the raw data. Additionally, a nonmetric multidimensional scaling (NMDS) was conducted to analyse the natural enemy community composition at each orchard and year in ant-excluded and ant-allowed treatments. NMDS analysis applied a square-root transformation and calculated the Bray-Curtis distances for the community matrix. NMDS analyses were conducted using R (R Development Core Team, 2014).

3. Results

A total of 176,000 arthropods belonging to 81 taxa were captured and identified in all samplings in the three orchards, including sticky traps and aspiration of the canopies. Of them, 53 taxa contained more than 40 individuals: 18 taxa of predators, 31 of parasitoids and 4 belonging to the fourth trophic level. These were the taxa included in the comparative analyses of abundance (Table 2). Among predators, the most abundant order was Neuroptera and the most abundant species were *Semidalis aleyrodiformis* Stephens and *Conwentzia psociformis* (Curtis) (both Neuroptera: Coniopterygidae). In the case of parasitoids, all of them belonging to the order Hymenoptera, the most abundant group was the superfamily Chalcidoidea, being *Aphytis chrysomphali* (Mercet) and *Cales noacki* Howard (Aphelinidae) the most abundant species (Table 2).

3.1 Abundance of parasitoids and predators

When comparing the abundance of specific species or arthropod taxa between treatments we observed different responses depending on the functional group and species of the natural enemy examined. From the 53 global comparisons of particular taxa of natural enemies between ant-allowed and ant-excluded trees, 21 (40%) showed a significant differences between treatments. When separated according to functional groups, 44% of the predator and 37% of the parasitoid taxa were affected by ants (Table 1). Further, in the comparisons obtained considering orchards individually, the percentage of taxa of natural enemies significantly affected by ants was 38% in orchard LH, 31% in orchard PP and 27% in orchard LG (Table 1).

In those cases where we detected significant differences between treatments we observed a general pattern. In the ant-allowed treatment predator abundance was significantly lower (seven species decreased and four increased in at least one orchard) whereas parasitoid abundance was higher (four species decreased and 18 increased in at least one orchard).

The abundance of natural enemies in ant-allowed and ant-excluded treatments also varied depending on the species of natural enemy (Table 1). This can be clearly seen for the following species by examining their seasonal population development in the ant-allowed and ant-excluded trees in the three orchards. The abundance of the generalist predators *Chrysoperla carnea* sensu lato (Stephens) (Neuroptera: Chrysopidae) and *Cardiasthetus* sp. (Heteroptera: Anthocoridae) was significantly lower in the ant-allowed trees. On the contrary, the abundance of parasitoids such as *Aphytis hispanicus* (Mercet) attacking *Parlatoria pergandii* Comstock (Hemiptera: Diaspididae), and *Anagyrus* sp. (Girault) (Hymenoptera: Encyrtidae) attacking pseudococcids, was significantly higher in the ant-allowed trees (Fig. 1).

Among true bugs (Heteroptera) the abundance of the myrmecomorphic *Pilophorus* sp. (Heteroptera: Miridae) was significantly higher in the ant-allowed treatment, especially in the orchard dominated by *L. grandis* (LG), whereas the abundance of other true bug species such as *Cardiasthetus* sp. (Heteroptera: Anthocoridae) or *Campyloneura virgula* (Herrich-Schäffer) (Heteroptera: Miridae) was significantly lower in the ant-allowed treatment (Fig. 2).

We captured several species belonging to the 4th trophic level (most of them identified as morphospecies) in the citrus canopies. *Marietta* sp. (Hymenoptera: Aphelinidae), hyperparasitoid of coccid parasitoids, and *Ablerus* sp. (Hymenoptera: Aphelinidae), hyperparasitoid of diaspidid partasitoids, were significantly more abundant in the ant-allowed treatment. The abundance of *Pachyneuron* sp. (Hymenoptera: Pteromalidae), hyperparasitoid of aphid parasitoids, was not significantly different between treatments. *Helorus* sp. (Hymenoptera: Heloridae), a parasitoid of chrysopid eggs, was less abundant in the ant-allowed treatment.

3.2 Species richness, diversity and community structure of predators and parasitoids

Overall, in the ant-allowed treatment the species richness (S) was significantly lower for predators and higher for parasitoids when compared with the ant-excluded trees. This impact of ants observed globally was, nevertheless, significant only in one orchard for predators and two orchards for parasitoids when examining the three orchards separately (Table 3).

The Shannon diversity index (H) was not different between the ant-allowed and ant-excluded treatments in the case of predators, whereas in the case of parasitoids diversity was significantly higher in the ant-allowed treatment both in the global analysis and in the three orchards analyzed individually (Table 3).

The multivariate test showed that the community of parasitoids changed significantly in the ant—allowed treatment in 2012 in the orchard LH (Table 4). In the other orchards and/or years the community structure was not different between treatments. Moreover, the ordination plot NMDS did not show a clear separation between ant-allowed and ant-excluded treatments in the community structure of the natural enemies (Fig. 3).

4. Discussion

Our results show that the community structure of predators and parasitoids was not significantly different between the ant-allowed and the ant-excluded treatments. However, when analyzing the effect of ants on the abundance of different species of natural enemies the results differ across species or taxa. The abundance of some species was lower in the ant-allowed treatment while for others it was unaffected or even higher. In general terms, we observed lower predator and higher parasitoid abundance in the ant-allowed treatment compared to the ant-excluded treatment. Most crucially, the species richness and diversity of parasitoids was higher in the ant-allowed treatment whereas the diversity of predators was not different between treatments.

4.1 Abundance of predators and parasitoids

It has been largely assumed that ant attendance offers hemipterans a protective service against parasitoids (Buckley, 1987; Flanders, 1951; Steyn, 1954). However, the abundance of most species of parasitoids in our study was either not affected by ants or was higher in the ant-allowed treatment. This effect seems to be related, at least in some cases, with the impact of ants on the parasitoids host populations and/or with the ability of the concrete species to cope with ant aggression. Often, honeydew producing pests are more abundant under ant protection and eventually this might explain the higher abundance of their parasitoids in the ant-allowed treatment. Parasitoids of honeydew producing pests, such as the soft scale parasitoids *Metaphycus helvolus* Compere, *Metaphycus flavus* Howard and *Encyrtus* sp. (Hymenoptera: Encyrtidae) or the mealybug parasitoid *Anagyrus* sp. were, in general, more abundant in the ant-allowed treatment, especially in the orchard LH. The overall abundance of Aphidiinae (Hymenoptera: Braconidae), parasitoids of aphids, was similar between treatments. However, when examining the response of different species within Aphidiinae the picture was different: *Lysiphlebus* sp. was significantly more abundant and *Aphidius* sp. was significantly less abundant in the orchard PP in the ant-allowed treatment. Völkl (1992) and Liepert and Dettner (1993) showed that ants attacked and killed *Trioxys angelicae* (Hymenoptera: Braconidae) while ignored *Lysiphlebus cardui* Marshall (Hymenoptera: Braconidae) due to chemical mimicry. On the other hand, Powell and Silverman (2010) reported that *Aphidius colemani* Viereck (Hymenoptera: Braconidae) were negatively impacted by *L. humile* and *Tapinoma sessile* (Say). In general, we may have two contrasting effects of ant attendance on parasitoids: ants disturb parasitoids but, on the other hand, increased populations of Hemiptera might result in potentially more hosts and eventually to higher parasitoid populations.

It was surprising to see that *Encarsia inquirenda* Silvestri and *A. hispanicus* (Hymenoptera: Aphelinidae), parasitoids of *P. pergandii*, that does not produce honeydew and therefore is not tended by ants, were more abundant in the ant-allowed treatment both globally and on each orchard individually (except in the orchard LG for *E. inquirenda*). Apparently, ant presence is associated with increased abundances of *P. pergandii,* as already found for other armored scales (Calabuig et al., 2013; Pekas et al., 2010; Yoo et al., 2013). Other parasitoid species of non-honeydew producers did not follow this trend. In the case of *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) and *A. chrysomphali*, parasitoids of *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae), we observed no differences in their abundance between treatments except in the orchard LH where the populations of *A. chrysomphali* were significantly higher in the ant-allowed treatment.

Predator abundance, at least for the most common species, was lower in the ant-allowed treatment. It is important to highlight the case of the chrysopids (green lacewings), *C. carnea* sensu lato and *C. septempunctata*. These species are considered important biological control agents in many agroecosystems (Senior and McEwen, 2001) and are among the most abundant predators in Mediterranean citrus orchards, preying upon a wide range of pests (Garcia-Marí, 2012). Our results are in agreement with other studies which have also found lower densities of chrysopids in ant-allowed treatments (James et al., 1999; Kaplan and Eubanks, 2002; McPhee et al., 2012; Vanek and Potter, 2010). Several authors reported aggressive behavior of different ant species against chrysopids (Bartlett, 1961; Vanek and Potter, 2010) or ant predation on chrysopid eggs (Dreistadt et al., 1986; Morris et al., 1998) which may result in lower chrysopid populations.

Regarding the impact of ants on coccinellids (Coleoptera) our results show great variability depending on the species examined. *Stethorus punctillum* Weise and *Rodolia cardinalis* Mulsant were less abundant in the ant-allowed treatment in orchard PP while *Delphastus catalinae* Horn and *Scymnus subvillosus* Goeze were more abundant in the ant-allowed treatment in orchard LH. The response of coccinellids to ant attacks differs between species (Jiggins et al., 1993) and some coccinellid species can cope with ant agression through morphological, behavioural or chemical adaptations. For example, Völkl and Vohland (1996) found higher populations of *Scymnus* sp. in ant attended resources due to the protective wax cover of the *Scymnus* larvae which allow them to predate upon honeydew producers tended by ants.

For the two most abundant species of predatory Heteroptera (true bugs) *Cardiasthetus* sp. and *Campyloneura virgula* we registered lower populations in the ant-allowed treatment. In an 8-year study, (Piñol et al., 2012b) also found lower abundance of predatory Heteroptera especially *Cardiasthetus fasciiventris* in ant-allowed trees. It is interesting to mention the higher populations of *Pilophorus* sp. in the ant-allowed treatment. These results are in agreement with other studies reporting a strong positive association between the ant *L. grandis* and *Pilophorus* sp*.* (Piñol et al., 2012b; Sanchez and Ortín-Angulo, 2012).This species exhibits mirmecomorphy that allows it to benefit from ant presence.

The abundance of some species from the 4th trophic level was also found to be different between the ant-allowed and ant-excluded treatments. This is apparently related with the abundance of their primary hosts. The hyperparasitoid of diaspidids *Ablerus* sp., which was present only in the orchard LH, was more abundant in the ant-allowed treatment, following the effect observed for its primary host *A. chrysomphali*. The hyperparasitoid *Marietta* sp. was also more abundant in the ant-allowed treatment, following the effect observed for the encyrtid parasitoids of coccids. The parasitoid of chrysopid eggs *Helorus* sp., on the other hand, was less abundant in the ant-allowed trees following the trend observed for its host. Several studies have demonstrated that some parasitoids benefit from ant attendance because ants may reduce hyperparasitism by disturbing hyperparasitoids (Sanders and Frank Van Veen, 2010; Völkl, 1992). On the other hand, intraguild predation caused by higher abundance of predators in ant excluded trees, which may predate upon parasitized hosts, may decrease the populations of some parasitoids and eventually hyperparasitoids (Kaneko, 2006, 2002; Novak, 1994).

4.2 Species richness, diversity and community structure of predators and parasitoids

Previous studies in several ecosystems showed a decrease of arthropod diversity as a result of ant activity (Human and Gordon, 1997; Wimp and Whitham, 2001). Nevertheless, it is difficult to compare these results with ours given that the previous studies focused on overall arthropod communities including different guilds such as herbivores. On the other hand, (Peng and Christian, 2013) found that weaver ants either had no impact or increased the diversity of natural enemies in cashew and mango trees. To our knowledge, the present study is the first to demonstrate a significant increase on the species richness as well as on the Shannon diversity index for parasitoids in the ant-allowed treatment.

Our results show that the community structure of predators and parasitoids on the citrus canopies were similar between treatments. Previous studies also found that ants did not affect the overall arthropod communities in vineyards (Chong et al., 2010), peaches (Mathews et al., 2009) or coffee (Philpott et al., 2008). Conversely, Piñol et al. (2012a), in an 8-year exclusion experiment, reported that ants changed the arthropod community in a citrus orchard in some years of the study. Differences in the results obtained in the different studies might be attributed to the different species of ants and/or the characteristics of the experimental orchards.

Despite *L. humile* having been described as an aggressive and very disruptive ant species for biological control (Markin, 1970), in our study we found no significant effects on the community structure of natural enemies. The same can be deduced for the native to the Mediterranean species in our study, *L. grandis* and *P. pallidula*. In fact, the impact of the three species on the community structure of natural enemies was quite similar. In a previous study it was found that the three ant species induced similar increases of the herbivore populations *A. aurantii* and *A. floccossus* (Calabuig et al., 2013).

In conclusion, our ant exclusion study reveals that ants in citrus are not associated with a dramatic decrease in natural enemy abundance or biodiversity at the community level. The impact of ants on the natural enemies depends mostly on the species of natural enemy; even closely related species showed different or opposite responses to ant activity. In spite of the species specific response of natural enemies, we detected a general tendency related to functional groups: predator abundance and diversity decreased whereas parasitoid abundance and diversity increased in the ant-allowed treatment. These results may have practical implications for biological pest control. Despite the fact that ants have no negative impact on the abundance and diversity of predators and parasitoids at the community level their impact on specific natural enemy species may explain the highest pest densities associated with ant presence in citrus (Calabuig et al., 2013; Dao et al., 2014; Pekas et al., 2010; Yoo et al., 2013). This would also highlight the importance of certain species, especially predators, rather than natural enemy assemblages on regulating pest populations as recently was demonstrated in olive groves in the Mediterranean (Paredes et al., 2015).

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Table 1. Studies examining the impact of ants on the diversity, community structure or abundances of natural enemies in different ecosystems.

Reference	Ecosystem	IMPACT OF ANTS		
		Diversity index	**Community structure**	**Natural enemy abundance**
James et al., 1999	1 Citrus orchard	n.d.*	n.d	Negative impact (in some seasons) on predatory beetles, lacewings, parasitic wasps and spiders.
Piñol et al., 2012	1 Citrus orchard	n.d	Impacted (includes herbivores)	Negative impact on several orders (analyzed at order level)
Philpott et al., 2008	3 Coffee farms	n.d	Impacted (includes herbivores)	No impact (order level).
Philpott et al., 2004	1 Coffee farm	n.d	Impacted depending on the ant species (includes herbivores)	Negative impact on Araneae and Coleoptera, but not other orders depending on the ant species.
Mody and Linsenmair, 2004	*Pseudocedrela kotschyi* trees	n.d	n.d	Negative impact on Araneae, Coleoptera, and Hymenoptera.
Peng and Christian, 2013	1 Cashew and 1 Mango orchard	Ants increased diversity of natural enemies in Cashew	n.d	No effect of ants in Mango and higher abundances in ant presence in Cashew (all natural enemies pooled).
Offenberg et al., 2005	Mangrove trees	n.d	No impact (only predators)	Negative impact on predators
Eubanks, 2001	3 Cotton fields	n.d	n.d	Negative impact on numerous predator species and Hymenoptera (grouped as wasps).
Human and Gordon, 1997	Variable vegetation types	No statistical analysis	n.d	Slightly negative impact on Araneae, Hemiptera and Cynipidae.
Wimp and Whitham, 2001	Poplar trees	Ants reduced diversity (includes all arthropods)	n.d	Negative impact on generalist predators; positive impact on aphid natural enemies.
Kaplan and Eubanks, 2002	4 Cotton fields	n.d	n.d	Negative impact on Chrysopids and Coccinellids,
Kaplan and Eubanks, 2005	2 Cotton fields	n.d	n.d	Negative impact on predators
Stewart-Jones et al., 2007	2 Apple orchards	n.d	n.d	Positive impact (all natural enemies pooled).
Chong et al., 2010	1 Vineyard	n.d	No impact	No impact
Vanek and Potter, 2010	Maple trees and Magnolias	n.d.	n.d.	Negative impact on Aranae, *Crysopa rufilabris* Burmeister and parasitic wasps (several species grouped) depending on the sampling date

*n.d. Not determined

Table 2. Total arthropods captured, arthropods captured in ant-allowed and ant-excluded trees (mean ± SE) and effect of ants, globally and for the three orchards separately, during two years, 2011 and 2012, in three citrus orchards.

Arthropods	Total arthropods	Principal prey	Global			*Ant effect / Orchard		
			Arthropods / sample		*Ant effect			
			Ant-allowed	Ant-excluded		LG	LH	PP
Neuroptera								
Chrysopidae								
Chrysoperla carnea (Stephens)	591	generalist	1.39 ± 0.19	2.70 ± 0.33	- -	- -	- -	- -
Chrysopa septempuctata Wesmael	39	generalist	0.07 ± 0.02	0.21 ± 0.04	- -	n.p.	- -	- -
Coniopterigidae								
Semidalis aleyrodiformis Stephens	29987	spider mites	107.39 ± 11.37	101.68 ± 10.46	0	0	0	0
Conwentzia psociformis (Curtis)	4395	spider mites	14.77 ± 3.65	15.84 ± 3.39	0	0	-	0
Coniopteryx sp.	65	spider mites	0.20 ± 0.04	0.25 ± 0.06	0	0	n.p.	0
Coleoptera								
Ragonycha sp.	235	generalist	0.59 ± 0.23	1.03 ± 0.32	0	0	n.p.	0
Cybocephalus sp.	131		0.67 ± 0.12	0.25 ± 0.05	+	0	+	0
Coccinellidae								
Scymnus subvillosus (Goeze)	1242	aphids	4.85 ± 0.41	3.83 ± 0.32	0	0	++	0
Rodolia cardinalis (Mulsant)	803	*Icerya purchasi*	2.54 ± 0.41	3.05 ± 0.50	0	0	0	- -
Delphastus catalinae Horn	626	whiteflies	2.22 ± 0.38	2.15 ± 0.49	++	0	0	0
Clitosthetus arcuatus Rossi	258	whiteflies	1.57 ± 0.13	2.18 ± 0.18	0	0	0	0
Rhizobius lophantae Blaisdell	89	whiteflies	0.36 ± 0.10	0.26 ± 0.10	0	n.p.	0	n.p.
Stethorus punctillum Weise	82	spider mites	0.26 ± 0.07	0.31 ± 0.05	- -	0	0	0
Diptera								
Platypalpus sp.	1338		3.07 ± 0.83	6.20 ± 1.88	0	0	0	0
Heteroptera								
Campyloneura virgula Herrich-Schäffer	745	generalist	2.04 ± 0.62	3.13 ± 0.71	- -	0	-	-
Cardiasthetus sp.	368	generalist	1.03 ± 0.15	1.53 ± 0.17	- -	- -	0	0
Ploearia sp.	78	spider mites	0.27 ± 0.07	0.28 ± 0.06	0	0	0	0
Pilophorus sp.	43	generalist	0.21 ± 0.04	0.09 ± 0.02	++	+	0	0
Hymenoptera								
Chalcidoidea								
Aphelinus sp.	127	aphids	0.21 ± 0.04	0.09 ± 0.02	0	0	0	0
Aphytis hispanicus (Mercet)	7534	*Parlatoria pergandii*	35.34 ± 6.08	17.47 ± 1.92	++	++	++	++
Aphytis melinus DeBach	11694	*Aonidiella aurantii*	39.96 ± 5.53	41.50 ± 7.12	0	0	0	0
Aphytis chrysomphali (Mercet)	50638	*Aonidiella aurantii*	167.94 ± 20.29	184.64 ± 21.83	0	+	++	0
Encarsia inquirenda (Silvestri)	3662	*Parlatoria pergandii*	15.21 ± 2.16	10.39 ± 1.22	++	- -	++	++
Encarsia sp. 1	59		0.26 ± 0.05	0.15 ± 0.03	++	0	++	0
Encarsia sp. 2	178		0.69 ± 0.34	0.55 ± 0.19	0	n.p.	0	n.p.
Encarsia sp. 3	1029		4.50 ± 0.63	2.71 ± 0.32	++	++	++	0
Cales noacki	18448	whiteflies	67.66 ± 8.16	61.01 ± 6.71	0	0	+	+
Marietta sp. **	313	coccid parasitoids	1.36 ± 0.32	0.83 ± 0.21	++	0	n.p.	++
Ablerus sp.**	645	diaspidid parasitoids	3.65 ± 0.96	0.90 ± 0.23	++	n.p.	++	n.p.
Eretmocerus sp.	102	whiteflies	0.35 ± 0.09	0.37 ± 0.09	0	n.p.	0	0
Citrostichus phyllocnistoides (Naranayan)	659	*Phyllocnistis citrella*	2.74 ± 0.86	1.86 ± 0.29	+	0	0	++
Metaphycus helvolus (Compere)	4355	coccids	18.83 ± 3.58	11.64 ± 1.56	++	0	++	++
Metaphycus flavus (Howard)	8005	coccids	29.43 ± 4.32	26.40 ± 4.03	0	0	++	0
Metaphycus lounsburyi (Howard)	75	coccids	0.35 ± 0.12	0.17 ± 0.05	0	n.p.	0	n.p.
Microterys nietneri (Motschulsky)	184	coccids	0.54 ± 0.11	0.73 ± 0.13	0	0	+	0
Anagyrus sp.	281	pseudococcids	1.43 ± 0.36	0.54 ± 0.11	++	++	++	++
Encyrtus sp.	62	coccids	0.33 ± 0.08	0.10 ± 0.04	++	n.p.	++	n.p.
Pachyneuron sp.**	65	aphid parasitoids	0.22 ± 0.08	0.23 ± 0.07	0	n.p.	0	n.p.
Mymaridae	4932	cicadellidae	19.65 ± 2.09	14.80 ± 1.81	++	+	0	+
Trichogramma sp.	75	lepidoptera	0.29 ± 0.05	0.23 ± 0.05	0	+	0	0
Ichneumonoidea								
Ichneumonidae	1038		3.70 ± 0.44	3.54 ± 0.43	0	++	0	0
Microgastrinae	587	lepidoptera	1.82 ± 0.23	2.26 ± 0.30	0	0	0	- -
Lysiphlebus sp.	93	aphids	0.42 ± 0.13	0.24 ± 0.08	0	0	0	++
Aphidius sp.	45	aphids	0.15 ± 0.04	0.17 ± 0.05	0	-	0	- -
Trioxys sp.	3451	aphids	11.94 ± 3.33	12.11± 3.09	0	0	0	0
Alysinae: Alysinii	172	leaf miners	0.59 ± 0.08	0.69 ± 0.08	0	0	0	0
Other Braconidae	270		1.01 ± 0.17	0.87 ± 0.14	0	0	0	0

Proctotrupoidea								
Helorus sp.**	309	crisopids	0.80 ± 0.14	1.34 ± 0.18	- -	0	- -	0
Chrysidoidea								
Chrysis sp.	62	hymenoptera	0.21 ± 0.09	0.22 ± 0.07	0	n.p.	0	n.p.
Cynipoidea	286		1.03 ± 0.17	0.97 ± 0.18	0	0	0	0
Ceraphronoidea								
Ceraphronidae	1083		4.26 ± 0.54	3.30 ± 0.34	++	++	0	n.p.
Megaspilidae	349		1.18 ± 0.18	1.25 ± 0.22	0	0	0	0
Platygastroidea								
Scelionidae	10897		39.73 ± 4.00	36.27 ± 3.11	0	0	0	0

* Repeated measures analysis of variance (ANOVA) was used, with treatment as fixed factor and time and block as random factors. In global analysis, orchard was set as random factor as well. The + indicates a slightly positive effect of ants on the abundance of the natural enemy (P<0.1); ++ indicates a significant positive effect of ants (P<0.05); - indicates a slightly negative effect of ants (P<0.1); - - indicates a significant negative effect of ants (P<0.05); n.p. indicates no presence of the natural enemy. LG: *Lasius grandis* orchard; LH: *Linepithema humile* orchard*;* PP: *Pheidole pallidula* orchard.

** Species belonging to the 4th trophic level.

Table 3. Impact of ants on Species richness (S) (mean ± SE) and Shannon diversity index (H) (mean ± SE) of predators and parasitoids, globally and for the three orchards separately.

Species richness (S)

	Predators					Parasitoids				
Orchard	Ant-excluded	Ant-allowed	d.f.	*F*	*P*	Ant-excluded	Ant-allowed	d.f.	*F*	*P*
LG	7.61 ± 0.37	6.96 ± 0.35	1,71	4.34	0.059*	11.02 ± 0.30	11.76 ± 0.33	1,71	5.50	0.037**
LH	8.42 ± 0.43	8.55 ± 0.42	1,56	0.05	0.828	13.54 ± 0.56	15.50 ± 0.60	1,56	14.08	0.003**
PP	7.35 ± 0.34	7.10 ± 0.38	1,75	0.76	0.401	11.58 ± 0.30	11.27 ± 0.31	1,75	0.78	0.396
Global	7.77 ± 0.22	7.44 ± 0.23	1,250	4.93	0.045**	12.03 ± 0.24	12.59 ± 0.28	1,250	14.02	0.003**

Shannon diversity (H)

	Predators					Parasitoids				
Orchard	Ant-excluded	Ant-allowed	d.f.	*F*	*P*	Ant-excluded	Ant-allowed	d.f.	*F*	*P*
LG	0.66 ± 0.06	0.62 ± 0.07	1,71	0.16	0.699	1.41 ± 0.04	1.51 ± 0.03	1,71	5.98	0.031**
LH	1.32 ± 0.08	1.45 ± 0.06	1,56	1.99	0.183	1.40 ± 0.05	1.54 ± 0.04	1,56	6.67	0.022**
PP	0.69 ± 0.06	0.60 ± 0.06	1,75	1.50	0.245	1.37 ± 0.05	1.45 ± 0.05	1,75	5.28	0.040**
Global	0.88 ± 0.04	0.84 ± 0.05	1,250	0.09	0.773	1.39 ± 0.03	1.49 ± 0.02	1,250	33.58	<0.001**

Repeated measures analysis of variance (ANOVA) was used, with treatment as fixed factor and time and block as random factors. In global analysis, orchard was set as random factor as well. ** indicates a significant effect of ants (P<0.05) and * indicates a marginally significant effect (P<0.1). LG: *Lasius grandis* orchard; LH: *Linepithema humile* orchard*;* PP: *Pheidole pallidula* orchard.

Table 4. Summary of the PERMANOVA results of the effect of ants on the predators and parasitoids communities in 2011 and 2012 in orchards LG, LH and PP.

		Predators		Parasitoids	
Year	Orchard	R^2	*P*	R^2	*P*
2011	LG	0.077	0.742	0.105	0.630
	LH	0.301	0.059	0.210	0.288
	PP	0.372	0.057	0.199	0.200
2012	LG	0.050	0.828	0.107	0.544
	LH	0.166	0.371	0.332	0.029**
	PP	0.395	0.085	0.043	0.943

Figure legends

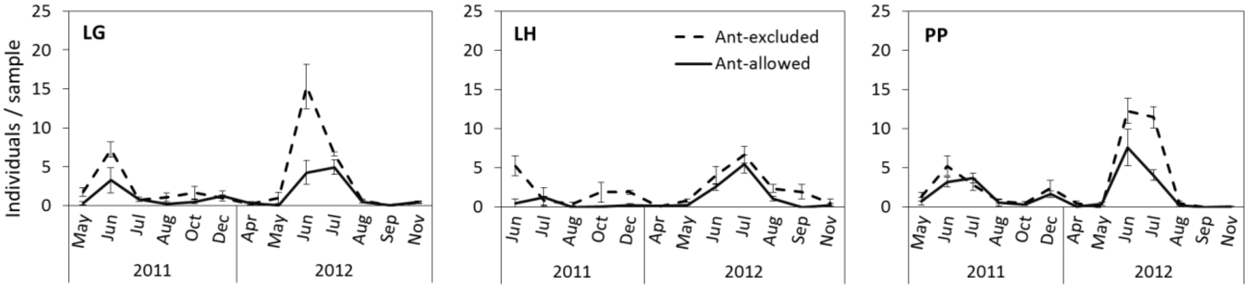
Figure 1. Abundance of *C. carnea* s.l., *Cardiasthetus* sp., *A. hispanicus* and *Anagyrus* sp*.* in ant-allowed and ant-excluded trees during 2011 and 2012. Abundance is measured as the mean number of individuals captured in one sticky trap and four aspirations (one/tree) in each experimental plot (each plot consisted in 16 trees and only the four central trees were sampled; each orchard contained 8 plots, 4 ant-allowed and 4 ant-excluded).

Figure 2. Abundance of *C. virgula* and *Pilophorus* sp. in ant-allowed and ant-excluded trees during 2011 and 2012. Abundance is measured as the mean number of individuals captured in one sticky trap and four aspirations (one/tree) in each experimental plot (each plot consisted in 16 trees and only the four central trees were sampled; each orchard contained 8 plots, 4 ant-allowed and 4 ant-excluded).

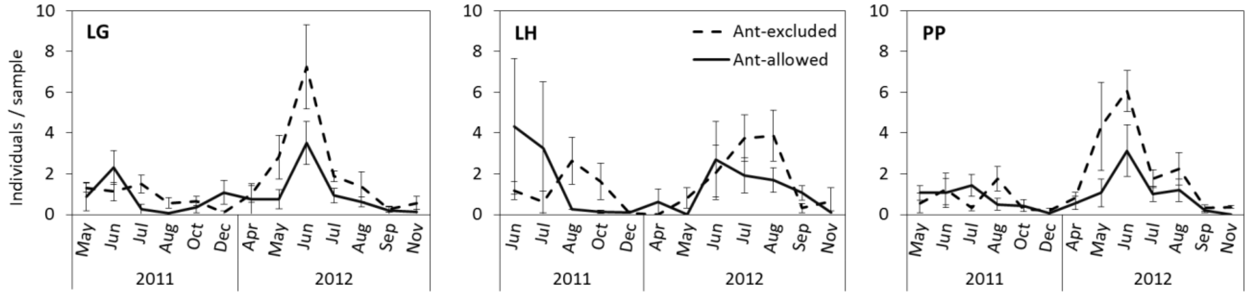
Figure 3. Nonmetric multidimensional scaling (NMDS) diagram of predators and parasitoids mean abundances in ant-allowed and ant-excluded trees in the three orchards and two years of the study. Straight lines connect ant-allowed and ant-excluded results for each case. Circles connect the results for each year of the study. LG*: Lasius grandis* orchard; LH: *Linepithema humile* orchard; PP: *Pheidole pallidula* orchard.

Fig. 1

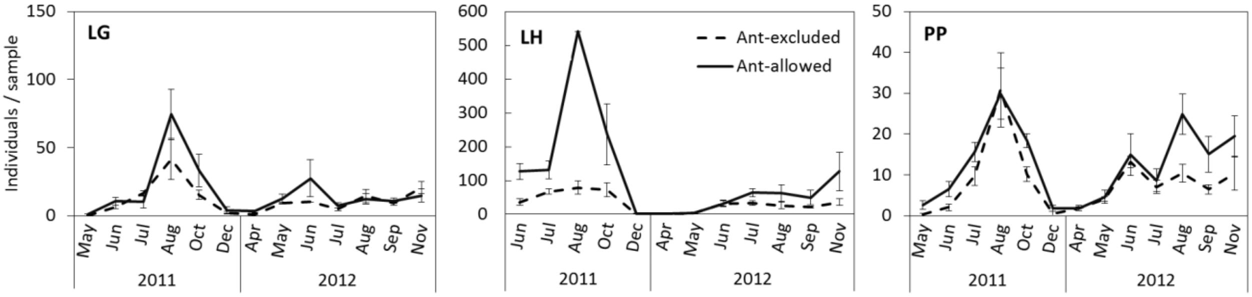
Chrysoperla carnea s.l.



Cardiasthetus sp.



Aphytis hispanicus



Anagyrus sp.

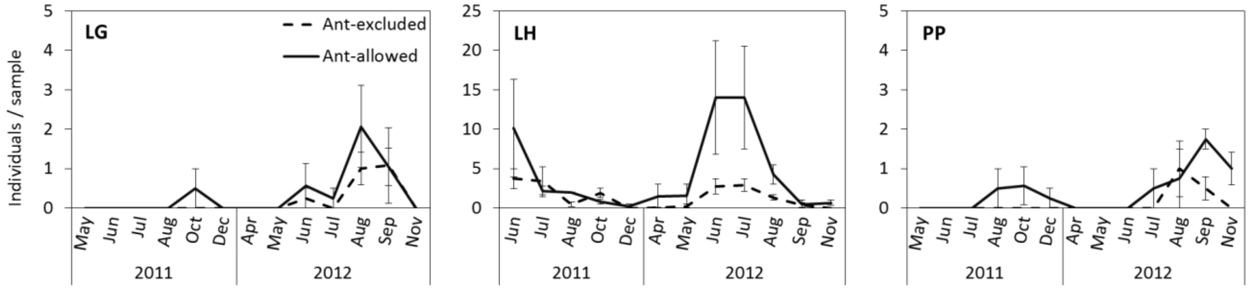
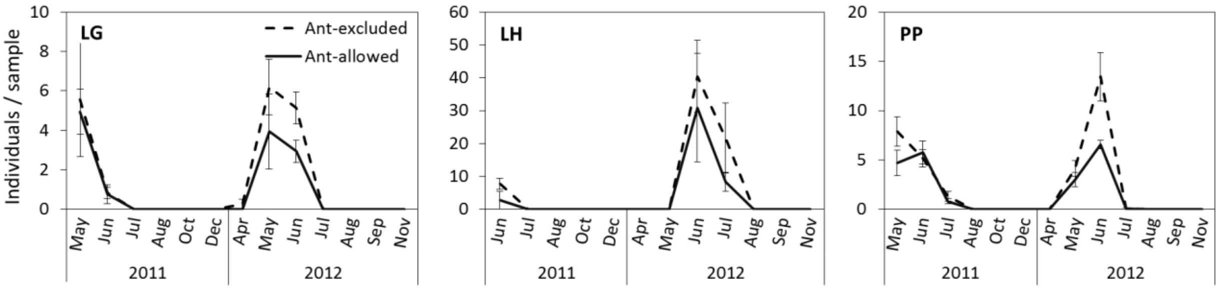


Fig. 2

Campyloneura virgula



Pilophorus sp.

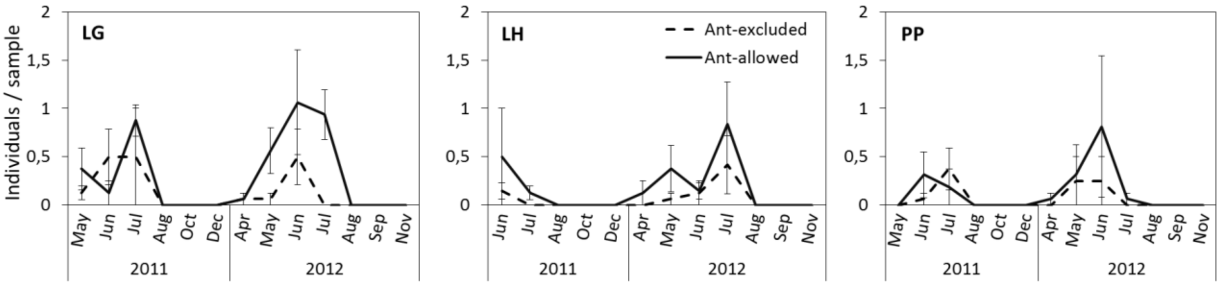
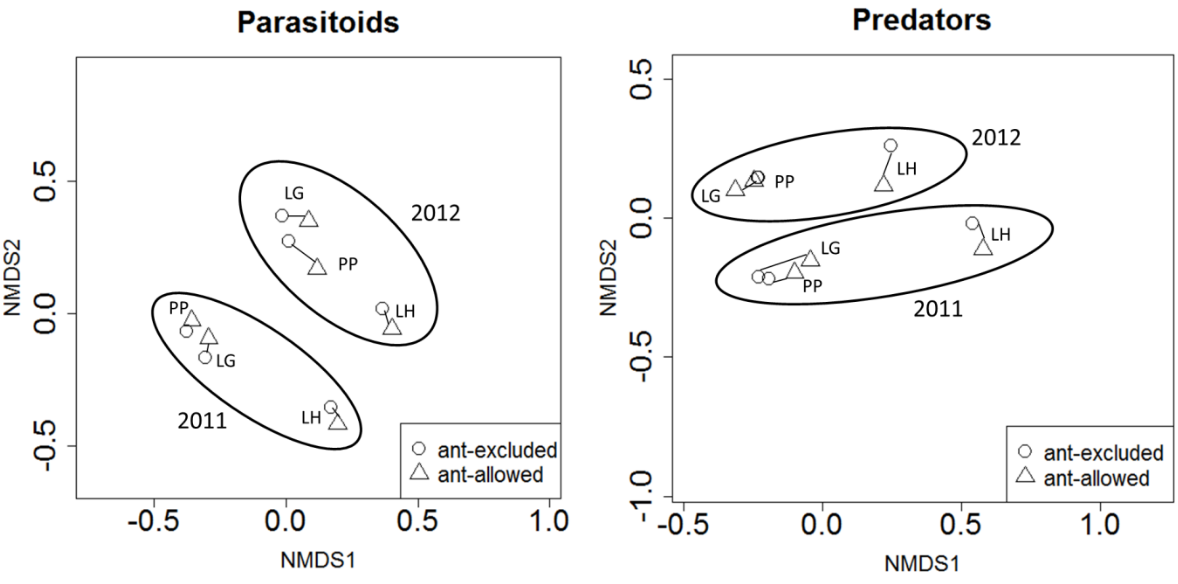


Fig. 3


Parasitoids
Predators
NMDS1
NMDS2
-0.5
0.0
0.5
1.0
-1.0
2011
2012
LG
PP
LH
ant-excluded
ant-allowed