Indirect Interactions Structuring Ecological Communities

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ABSTRACT

Ecological communities are collections of species bound together by their influences on one another. Community structure, therefore, refers to the way in which these influences are organised. As a result, ecologists are mainly interested in the factors driving the structure, functioning, and persistence of communities. The traditional focus, however, has been on the feeding relationships among species (direct trophic interactions), whereas relationships mediated by a third species or the environment (indirect interactions) have been largely overlooked. I investigated the role of indirect interactions in structuring communities through a series of field experiments in a diverse assemblage of arthropods living on a Brazilian shrub species. I experimentally reduced the abundance of the commonest galler on the shrub and found that the perturbation resonated across the food web, affecting its structure and robustness. Since there was no potential for these effects to be propagated directly or indirectly via the documented trophic links, the effects must have spread non-trophically and/or through trophic links not included in the web. Thus, I investigated non-trophic propagation of effects in the system. I demonstrate that hatched galls of the commonest galler, which serve as habitat for other species, can mediate non-trophic interactions that feedback to the galler modifying its interactions with parasitoids and inquiline aphids. I performed further manipulative experiments, excluding ants, live galls and hatched galls, to reveal mechanisms for the non-trophic interaction modifications observed in this system. Finally, I explored how non-trophic interaction modification could affect the structure and stability of a discrete ecological community in the field. I investigated how the densities of certain pairs of groups relate to each other, and how their relationship changes in relation to a third group. Then, I assembled an "effect network" revealing, for the first time in an empirical community, a hidden web of non-trophic indirect interactions modifying the direct interactions and modifying each other. Overall, the thesis presents evidence that communities are strongly interconnected through non-trophic indirect interactions. This is one of the first empirical demonstrations of the context-dependent modification of interactions via non-trophic interactions. However, determining the mechanisms behind such interaction modifications may be unfeasible. Understanding how the observed effects relate to community structuring requires shifting our focus from bipartite interaction networks to a more holistic approach.

STATEMENT OF AUTHOR CONTRIBUTION

For chapters 1 and 2, which were written as papers for publication, I conceived and designed the experiments, collected and analysed the data and wrote the initial manuscript. Dr R J Morris provided guidance on the design and data analysis, and all co-authors have edited the manuscripts for publication.

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GENERAL INTRODUCTION

GENERAL INTRODUCTION

Ecological communities are collections of species bound together by their influences on one another (Begon et al. 2006). Community structure, therefore, refers to the way in which these influences are organised. Ecologists are mainly interested in the factors driving the structure, functioning, and persistence of communities. For instance, how hundreds of species are organised in highly complex and apparently persistent communities (Lewis 2010, Andersen et al. 2012). This knowledge is essential to predict and mitigate human-induced environmental perturbations, such as habitat degradation and fragmentation, alien species invasion, and climate change (Lewis et al. 2002, Tylianakis et al. 2007, Memmott 2009).

Trophic interactions

Species can affect each other in many different ways. The most obvious and readily observable influences are direct interactions, those involving a physical interaction between two species, such as when species consume, interfere, or physically benefit another. On the other hand, indirect trophic interactions require the presence of a third species as an intermediate (Wootton 1994). For instance, in a trophic cascade, a top predator in a food web alters the density and/or behaviour of their prey, and thereby indirectly affects consumption on the next lower trophic level (Estes & Palmisano 1974). Indirect trophic interactions play an important role in community structuring and were usually not accounted for in traditional studies (Bukovinszky et al. 2008). More recently, however, indirect effects have received growing attention in ecological research and their role in structuring ecological communities has been experimentally demonstrated (Tompkins et al. 2000, Morris et al. 2004, Sanders et al. 2015). For instance, Sanders et al. (2015) provide experimental evidence that the positive indirect effects that consumer species have on each other by reducing competition among their respective resource species can help maintain consumer species diversity. In addition, indirect interactions may be responsible for unexpected results of experimental manipulations of direct interactions (Doak et al. 2008) – e.g, experimentally reducing the abundance of a species affects other unrelated species in a food web (chapter 1). In fact, many of the effects that were interpreted in the past as being a result of competition may be caused by other indirect interactions (Holt 1977). For example, the negative effect of one herbivore on another may produce an outcome that resembles resource competition but is, in reality, mediated by a shared predator, a process termed apparent competition (Holt 1977, Morris et al. 2004). Thus, it has become clear that the structure and dynamics of ecological communities cannot be fully understood without taking indirect interactions into consideration.

Food webs

Ecologists have used food webs, the relationships of who eats whom in ecological communities, to study the role of structural complexity in the dynamics and stability of ecosystems (Solé & Montoya 2001, Dunne et al. 2002, Montoya et al. 2006, Rooney et al. 2006). Earlier studies were limited to constructing traditional qualitative webs, showing only binary information on presence or absence of particular trophic links between species

(Schönrogge 1995), or semi-quantitative webs that also include relative attack rates of different enemies (Stone & Schönrogge 2003). However, these webs do not describe emergent food web structure, or how often species interact with each other (Kaartinen & Roslin 2012).

More recently, ecologists started to generate larger, highly taxonomically resolved (Bascompte & Melián 2005, Montoya et al. 2006) and quantitative webs, showing species' abundances and frequency of interactions (Müller et al. 1999, Lewis et al. 2002, Kaartinen & Roslin 2012). This permits distinguishing the interactions that are frequent from those that are casual (Lewis et al. 2002). New metrics based on information theory have recently been generated and applied to quantitative food webs that enable a quantitative measure of their structural attributes (Bersier et al. 2002). These quantitative metrics are calculated by weighting each interaction by its abundance. For instance, Weighted quantitative connectance measures the weighted ratio of realized trophic links to all possible links within the web (quantitative linkage density / number of species; Tylianakis et al. 2007); and Interaction evenness measures uniformity of energy flows and is based on Shannon's evenness of network interactions (Tylianakis et al. 2007).

Producing fully quantified webs nonetheless requires great sampling effort (Memmott & Godfray 1994, van Veen et at. 2006), and therefore it is usually easier to focus on subsets of food webs, such as guilds of strongly interacting species (Lewis et al. 2002). As a result, food webs have mostly comprised host-parasitoid interactions. Herbivore insects and their parasitoid show a high degree of specialisation to their resource species and thus constitute well-defined and easy to sample models for the construction of quantitative food webs (Morris et al. 2004, van Veen et al. 2005, 2006, Kaartinen & Roslin 2012). Also, because parasitoid attack is not immediately destructive to their host insect, it is possible to quantify the frequency of interactions (van Veen et al. 2006). Gall-inducing and leaf-mining insects have the extra advantage of being sessile, allowing in situ manipulation and monitoring of populations over time. They are also highly specialised to the host plant and can be taxonomically identified through external morphology (Shorthouse & Rohfritsch 1982, Carneiro et al. 2009).

Non-trophic interactions

Although the traditional focus in community ecology has been mostly on the feeding relationships among species (direct trophic interactions), not all interactions involve feeding relationships (Strauss 1991, Kéfi et al. 2012, Pocock et al. 2012). Pollinators and seed dispersers although feeding on plant products have direct positive effects on plants that are not feeding-related - seed and pollen transportation. Furthermore, two herbivores may indirectly affect each other by modifying the quality of their shared host plant, via induction of physical or chemical plant defences (Vandermeer 1969). As a result, direct and indirect non-trophic interactions are widely spread in natural systems (NTI; Wilson 1980, Wootton 1994) and can also play an important role in determining the structure of communities (Strauss 1991, Kéfi et al. 2012).

However, NTI are not limited to effects mediated via a third species, as species can also indirectly affect one another abiotically via numerous shared physical conditions (eg, temperature, pH, salinity) and resources (eg, space, nutrients, light; Wilson 1980, Wootton 1994). Galling insects, for instance, can affect litter decomposition and nutrient release, increasing the availability of nitrogen (Hunter 2001, Schweitzer et al 2005). Galls can also serve as habitat for numerous organisms, including other insects, spiders, mites and fungi, providing shelter and access to other resources (Martinsen et al 2000, Ohgushi 2005, Crawford et al 2007). Ohgushi et al. (2005) describe an indirect interactions web including NTI, in which gall initiation enhanced shoot growth, increasing the density of leaf-rolling caterpillars. After caterpillars abandoned the leaves, more shelter was made available for aphids. An increased number of ants tending aphids, in turn, reduced the population of a specialist beetle. All these NTI took place, even though there was no direct contact among most of the species.

Integrating networks

It is clear that the species can influence each other in a myriad of interactions mediated trophically and non-trophically (Strauss 1991, Wootton 1994, Morris et al. 2004, Sanders et al. 2015). Each species has the potential to indirectly affect many others through their activities - e.g., soil aeration by earthworms, construction of dams by beavers; and "byproducts" - e.g., soil fertilisation by excrements, release of allelopathic compounds by plants (Wilson 1980, Wootton 1994, Ohgushi 2005). Therefore, the focus on specific trophic interactions neglects much of the information on the role of species in structuring communities (Ohgushi 2008, Kéfi et al. 2015). More recently, networks including NTI such as mutualistic webs (e.g., plant – pollinator) have also been constructed (see Ings et al. 2009). However, the interactions studied in these distinct types of network are not independent of other interactions in the community, as in nature networks are interlinked (Ings et al. 2009, Knight et al. 2005). As a result, there have been some recent suggestions that future research should work towards building a more comprehensive ecological network theory, integrating trophic and non-trophic interactions (Fontaine et al. 2011, Kéfi et al. 2012). Both empirical and theoretical efforts to propose new frameworks and to map NTI onto food webs have shown that there is much to gain from merging different types of interactions into a single network (Knight et al. 2005, Fontaine et al. 2011, Kéfi et al. 2012, 2015), although to date progress is still limited.

Interaction Modification

Another important and more challenging aspect to be considered in analyses of networks is that interactions can vary in space and time (Chamberlain et al. 2014, Poisot et al. 2015). Besides trophic interactions, there has also been a focus in classical ecology on pairwise interactions. However, ecological communities are not static objects and the fact that two species interact in one way at given space and time do not mean that they will always interact in that same way (Havens 1992). Environmental conditions and community composition will influence the magnitude, sign and even the occurrence of interactions (Menge et al. 1994, Callaway et al. 2002, Chamberlain et al. 2014, Poisot et al. 2015) with important implications for species coevolution (Thompson 2005). Furthermore, there are higher-order interactions, where a pairwise interaction is modulated by a third species - or an abiotic factor, resulting in an indirect interaction (Wootton 1994, 2002). Such high-order interactions may involve trophic or non-trophic interactions (Bairey et al. 2016).

Empirical and theoretical studies suggest that interaction modification cannot be ignored in predicting community responses to perturbations (Fontaine et al. 2011, Kéfi et al. 2012, Bairey et al. 2016; see also chapter 2). NTI are likely to have a key role in stabilising multi-trophic communities (increasing species persistence) by modifying the strength of trophic interactions, but empirical evidence is still lacking. Computational models and laboratory experiments indicate that NTI can reduce chances of species extinction, for instance, by preventing predators from overexploiting their prey (McCann et al. 1998, McCann 2000, Bonsall et al. 2002, Hammill et al 2015), or by reducing competition among resource species and allowing the coexistence of consumer species (van Veen et al 2005).

Overall Aims

My thesis aimed to address the gaps in knowledge concerning the interplay between trophic and non-trophic indirect interactions in structuring communities. In particular, I aimed to investigate under natural conditions the mechanisms of trophic and non-trophic indirect interactions taking place at the community level. In contrast to previous studies that investigated particular pathways of indirect interactions in isolation (Knight et al. 2005, van Veen et al 2005, Hammill et al 2015). In addition, I tested the role of NTI in interaction modification and ultimately in species persistence in multi-trophic communities. To do this I chose to work on a diverse and self-contained assemblage of arthropods living on a shrub species in a Brazilian savannah habitat.

Study system

The study system I worked on is a self-contained assemblage of arthropods living on the shrub species *Baccharis dracunculifolia* D.C. (Asteraceae). The plant hosts a species-rich fauna of insect herbivores and has been extensively used as a study model in ecological research. As a result, several interactions have been empirically demonstrated to date involving the plant species and associated insects (interactions described below; Collevatti & Sperber 1997, Fernandes et al. 1999, Espírito-Santo & Fernandes 2002, Fagundes et al. 2005, Neves et al. 2011). Many of them are multitrophic and indirect interactions. This facilitated the design of the experiments carried out in my thesis. Additionally, *B. dracunculifolia* hosts the largest fauna of galling insects in the Neotropics, with 17 species (Fernandes et al. 1996). Galling insects usually compose good model system for being easy to identify and record, allowing *in situ* manipulation and monitoring of populations over time. They are also highly specialised to the host plant and can be taxonomically identified through gall external morphology (Shorthouse & Rohfritsch 1982, Carneiro et al 2009). Also, since parasitoid attack is not immediately destructive to their host insect, it is possible to quantify the frequency of interactions (van Veen et al. 2006).

The plant species B. dracunculifolia is a perennial, evergreen, dioecious shrub, 2-3 m in height, which is widely distributed across southern and central South America (Espírito-Santo et al. 2003). B. dracunculifolia frequently forms well-defined patches 18 to 12,000 m^2 in area (Collevatti & Sperber 1997). It has a key role in natural succession and regeneration (Fernandes et al. 2016) and is, therefore, important in terms of biodiversity and ecosystem functioning. The plant species hosts many species of free-feeding herbivores, mostly Hemiptera, Coleoptera and Orthoptera, and also many predator species including the orders Araneae, Coleoptera, Matodea, and Hymenoptera. Baccharopelma dracunculifoliae (Sternorrhyncha: Psyllidae) is by far the commonest galler (Araújo et al. 1995), creating up to 83% of galls (chapter 1). It induces a gall in the midrib of the leaf, which bends over itself until the borders are joined, forming an elliptical, green, glabrous, single-chambered gall that usually harbours up to four nymphs (Lara & Fernandes 1994). The gall is not completely sealed as the leaf borders are joined but not fused. The galls remain attached to the plant after dehiscence and gradually become dry and woody (Espírito-Santo & Fernandes 1998). Both hatched and non-hatched galls of B. dracunculifoliae are occupied by many inquiline invertebrates, such as ants, spiders, aphids, etc. (Collevatti & Sperber 1997, Espírito Santo & Fernandes 2002). At least ten parasitoid species have been reared from galls of B. dracunculifoliae (chapter 1) and parasitism rates are around 45% (Espírito-Santo & Fernandes 2002). When the parasitoids emerge, they leave a characteristic exit hole on the gall wall (Espírito-Santo et al. 2004), facilitating aphid colonisation of parasitised galls (chapter 3).

Beyond the parasitoids, the gallers are also host to inquilines (species that occupy a living space produced by another species – e.g., a gall). The additional interactions with the inquilines on top of the parasitoids, provide a further layer of interactions, making the system even more complex. The aphid *Uroleucon tucumani* (Sternorryncha: Aphididae) is by far the most frequent inquiline and can indirectly kill the nymphs of the gall maker (Collevatti & Sperber 1997, Fagundes et al. 2005, chapter 3). This aphid species also feeds and reproduces on the apical meristems of the host plant, forming dense colonies that produce honeydew (sugary secretions). *U. tucumani* attracts at least 15 species of ants, which tend and protect them in a trophobiotic relationship (Fagundes et al. 2005, Neves et al. 2011, MB *pers. obs*). It has been found that the presence of ants reduces the number of *B*. *dracunculifoliae* nymphs per gall, and the presence of aphids reduces *B*. *dracunculifoliae* gall size because aphids compete with the galler for sap assimilates and young leaves in terminal buds (Fagundes et al. 2005). Neves et al. (2011) observed that the presence of ants and aphids on *B*. *dracunculifolia* decreased the abundance of other free-feeding herbivores and that the presence of aphids decreased plant shoot growth. Ants tending aphids can have a direct negative impact on herbivores (Fernandes et al. 1999). However, the aphids on their own can also reduce the abundance of fluid-sucking and chewing insects due to exploitation competition or by altering the nutritional quality of the host plant (Fay et al. 1996, Larson & Whitham 1997).

The focal community represents a very appropriate model system for the study of indirect interactions under natural field conditions. The patchy distribution of the host plant combined with a diverse and fairly specialised fauna of arthropods creates a discrete and yet highly self-contained multi-trophic community. These characteristics not only facilitate the manipulation and monitoring of species densities but also increases the chances of observing their effects, as they are more likely to be a result of local ecological processes, rather than being entangled with external processes (e.g., compensatory migration). In addition, contrary to experimental manipulations under laboratory conditions, where sets of interacting species are studied in isolation, in this system the propagation of systemic indirect effects is possible.

Thesis outline

The chapters are organised as stand-alone pieces of research, so each chapter has its own introduction and discussion.

In the **first chapter**, I investigate the role of direct and indirect interactions on food web structure and robustness (tolerance to species loss). Theoretical models from previous works suggest that the loss or reduction in abundance of individual species can lead to secondary and cascading extinctions (Saavedra et al. 2008, Staniczenko et al. 2010). I experimentally manipulated quantitative host-parasitoid food webs to reduce the abundance of *Baccharopelma dracunculifoliae* (Sternorrhyncha: Psyllidae), the commonest gall-making species associated with *B. dracunculifolia*. I investigated direct effects of the manipulation on parasitoids attacking the manipulated galler, as well as indirect effects on ten other galler species and 50 parasitoid species associated with them. I used metrics of food web structure and robustness (tolerance to species loss) to compare webs from control and manipulated plots.

In **chapter two**, I examined the non-trophic propagation of effects in the same system. Theory suggests that NTI can be a major mechanism of community stability and persistence, but empirical data are scarce (Strauss 1991, Kéfi et al. 2012). Here I investigated whether NTI initiated by ecosystem engineering - physical changes to the biotic or abiotic environment caused by a species - can feedback to the engineer, modifying the magnitude and direction of its interactions with other species. Gall induction can be viewed as ecosystem engineering since both live and hatched galls serve as habitat for other species, particularly aphids. Thus, in a field experiment, I generated treatments with reduced or elevated ecosystem engineering by removing or adding hatched galls.

In **chapter three** I aimed at going beyond purely demonstrating who affects who and how intensely, and to better understand the role of NTIs in community structuring. Specifically, I aimed to identify the mechanisms through which NTI can modify interactions and affect the structure and stability of a relatively simple and discrete ecological community in the field. I performed further manipulative experiments on the same system to test hypotheses on the mechanistic basis for the NTI previously observed in previous chapters. I investigated: (i) how reducing the abundance of the commonest galler indirectly affected food web structure and robustness (chapter 1); (ii) how aphids interfere with parasitism rate of the dominant galler (chapter 2); (iii) how hatched galls interfere with parasitism rate of the dominant galler (chapter 2), and; (iv) whether hatched galls, aphid colonies, and ants tending aphids interfere with abundance and parasitism of a second galler (chapter 1).

Finally, in **chapter four**, I built an "effect network" portraying trophic and non-trophic interactions. Ecologists have become increasingly aware of the importance of non-trophic interactions in ecosystem structuring and dynamics (Fontaine et al. 2011, Kéfi et al. 2012). However, there have been little empirical investigation of their significance at the level of whole ecological communities. I removed live and hatched galls of the dominant species, as well as ants, from different plants, and quantified the changes in densities of several other species or guilds (groups) as well as changes in direct interactions involving the galler, such as parasitism, and inquilinism by aphids. This allowed the construction of an "effect network", with links categorised into two main types: node modulation, which are pairwise trophic and non-trophic interactions (effect on densities); and link modulation, which are three-way interactions (interaction modification) or four-way interactions (modification of an interaction modification).

Finally, in the **General Discussion**, I summarise my results and highlight common themes emerging from the four chapters. I end by highlighting future directions for research in this area.

CHAPTER 1

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CHAPTER 1: Experimentally reducing species abundance indirectly affects food web structure and robustness

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Running headline: Perturbations propagate through food webs

Abstract

1. Studies on the robustness of ecological communities suggest that the loss or reduction in abundance of individual species can lead to secondary and cascading extinctions. However, most such studies have been simulation-based analyses of the effect of primary extinction on food web structure.
2. In a field experiment we tested the direct and indirect effects of reducing the abundance of a common species, focusing on the diverse and self-contained assemblage of arthropods associated with an abundant Brazilian shrub, *Baccharis dracunculifolia* D.C. (Asteraceae).
3. Over a 5-month period we experimentally reduced the abundance of *Baccharopelma dracunculifoliae* (Sternorrhyncha: Psyllidae), the commonest galling species associated with *B. dracunculifolia*, in 15 replicate plots paired with 15 control plots. We investigated direct effects of the manipulation on parasitoids attacking *B. dracunculifoliae,* as well as indirect effects (mediated via a third species or through the environment) on ten other galler species and 50 associated parasitoid species.
4. The experimental manipulation significantly increased parasitism on *B. dracunculifoliae* in the treatment plots, but did not significantly alter either the species richness or abundance of other galler species. Compared to control plots, food webs in manipulated plots had significantly lower values of weighted connectance, interaction evenness and robustness (measured as simulated tolerance to secondary extinction), even when *B. dracunculifoliae* was excluded from calculations.

5. Parasitoid species were almost entirely specialised to individual galler species, so the observed effects of the manipulation on food web structure could not have propagated via the documented trophic links. Instead, they must have spread either through trophic links not included in the webs (e.g., shared predators) or non-trophically (e.g., through changes in habitat availability). Our results highlight that the inclusion of both trophic and non-trophic direct and indirect interactions is essential to understand the structure and dynamics of even apparently discrete ecological communities.

Key words: Cerrado, experimental manipulation, galling insects, indirect interactions, networks, non-trophic interactions, parasitoid wasps, parasitism, species loss, rupestrian grasslands

Introduction

Understanding the impact of species declines and extinctions on communities and ecosystems has become a major goal in ecology and conservation biology (Daily 1997, Chapin *et al*. 2000, Ripple et al. 2015). Simulations suggest that removing species from food webs at random has less impact on communities than the selective removal of species with more trophic connections (Dunne et al. 2002). Thus, the consequences of declines in biodiversity depend on community structure and complexity (Albert et al. 2000, Downing & Leibold 2002).

The tolerance of a food web to secondary extinctions resulting from species loss can be measured as its "robustness" (Dunne et al. 2002; 2004, Memmott et al. 2004, Pocock et al. 2012). Studies on the robustness of ecological communities suggest that the loss of individual species can lead to secondary and cascading extinctions (Shin et al. 2004, Ebenman et al. 2004, Dobson et al. 2006, Saavedra et al. 2008, Staniczenko et al. 2010). To date, most such studies have been simulation-based analyses of the effect of primary extinction, assuming bottom-up control, i.e., species are considered extinct when their resource species are no longer available (Sanders & van Veen 2012). However, cascading secondary extinctions may happen as a result of several other direct and indirect, positive and negative effects, such as top-down and horizontal indirect effects (Eklof et al. 2006, Curtsdotter et al. 2011, Sanders et al. 2015). Indirect trophic and non-trophic interactions play a major role in community structuring and have not usually been accounted for in traditional topological analyses of food webs (Strauss 1991, Bukovinszky et al. 2008). More recently, however, indirect effects such as apparent competition have received growing attention and their importance has been demonstrated experimentally (Tomkins et al. 2000, Morris et al. 2004). Non-trophic interactions may also have considerable influence on community structure (Strauss 1991, Kéfi et al. 2012), but their relative importance is yet to be revealed (Sutherland et al. 2013).

Using experimental mesocosms containing three predators and three prey sharing a host plant, Sanders, Kehoe & van Veen (2015) recently demonstrated that initial extinctions of predators can trigger further predator extinctions due to indirect population-dynamic effects. However, equivalent large-scale, long-term field manipulations of species-rich quantitative food webs are lacking (but see Morris et al. 2004, Biles et al. 2003, Díaz et al. 2003, Kaartinen & Roslin 2012). Such studies would allow for the identification of processes following species exclusion such as compensation by other species (Schläpfer & Schmid 1999, Loreau 2000, Díaz & Cabido 2001, O'Connor & Crowe 2005) and "rewiring", i.e., shifts in the incidence or frequency of interactions (Tylianakis et al. 2007, Kaiser et al. 2010).

We conducted a replicated field experiment to test the effect of reducing the abundance of a very abundant species on food web structure and robustness in a diverse but discrete field system focused on a major plant species and its species-rich assemblage of associated arthropods, in the Brazilian Cerrado. We manipulated quantitative host-parasitoid food webs by reducing the abundance of a dominant insect herbivore (a gall-making insect) and tested the prediction that this would result in direct effects on parasitoids attacking the manipulated host, as well as indirect effects on other galler species and their parasitoids. We also tested the prediction that metrics of food web structure and robustness (tolerance to species loss) would differ between control and manipulated plots.

Methods

Study Site

This study was conducted at Serra do Cipó, in Minas Gerais state, southeast Brazil. This region is in the southern portion of the Espinhaço Mountain Chain, in the Cerrado biome and is characterized by quartzitic soils covered by rocky grasslands, with predominance of herbs and shrubs (Fernandes 2016). It has a Cwb Köppen climate type, with dry winters and rainy summers (Schulz & Machado 2000). The average annual rainfall is between 1250 and 1550 mm, and the average temperature ranges from 18 to 19º C (Madeira & Fernandes 1999). The study sites were located alongside a 14 km section of highway MG-010 (from 19°17' - 19°15'S and 43°35' -43°31'W). Studied sites varied from 1127 to 1365 m elevation.

The Baccharis system

The host plant species *Baccharis dracunculifolia* is a perennial, evergreen, dioecious shrub, 2-3 m in height, which is widely distributed across southern and central South America (Barroso 1976, Espírito-Santo et al. 2003). *Baccharis dracunculifolia* frequently forms well-defined patches 18 to 12,000 m^2 in area (Collevatti & Sperber 1997). It has a key role in natural succession and regeneration (Fernandes et al. 2016) and is therefore important in terms of biodiversity and ecosystem functioning. Among the 17 species of gall inducing insects recorded on *B. dracunculifolia* in multiple locations (Fernandes et al. 1996, 2014), *B. dracunculifoliae* is the commonest (Araújo et al. 1995). It induces a gall in the midrib of the leaf, which bends over itself until the borders are joined, forming an elliptical, green, glabrous, single-chambered gall (Lara & Fernandes 1994).

Experimental design

We selected 30 discrete clusters of *B. dracunculifolia*, each comprising approximately 15 to 20 individuals growing ≤5 m apart. These clusters, hereafter referred to as plots, were distributed in pairs 10-50 m apart from each other, and from other shrubs of the same species, across 15 spatial blocks ≥280 m apart, and were randomly allocated to control and treatment within each block. The treatment consisted of removing galls induced by *B. dracunculifoliae*, the commonest galler species found on *B. dracunculifolia,* by direct collection of the galls (which are essentially modified leaves). An equivalent number of non-galled leaves were collected from the control plots. The host plants have huge numbers of very small leaves and only a tiny fraction of them were removed (<5%, MB unpublished data), making any change in leaf availability to gallers negligible. Since new galls could be induced over the monitoring period, the treatments were maintained by excluding newly induced galls every month, one or two weeks before sampling the galler-parasitoid community.

Collection of quantitative food web data

We collected quantitative information on the density of each galler species attacking *B. dracunculifolia* and the proportion of individuals of each species parasitised by various parasitoid species. We estimated the abundance of each galler species three times over five months (September, October and December 2013), one or two weeks after the removal treatment (described above). We collected and counted all full-sized or close to full-sized galls on five 0.5 m lengths of branch haphazardly chosen around the crown of each of five individuals of *B. dracunculifolia* within each plot. The sampled shrubs were marked and were not sampled in the subsequent month. Galler species were identified in the field based on external morphology and the plant organ affected (see Fernandes et al. 1996). Nearly all galler insects are specific to a single plant species and organ, and gall structure is persistent, meaning that the external morphology is consistent across individuals of each species (Rohfritsch & Shorthouse 1982, Floate et al. 1996). Thus, gall morphospecies are commonly used as a proxy for species of galler insects (Fernandes & Price 1988, Carneiro et al. 2009).

The collected galls were reared in the laboratory by placing the leaves or stems containing galls of the same morphospecies together in 250 ml plastic pots covered with nylon mesh (0.04mm net, 80% light penetration) and a perforated plastic lid (Espírito-Santo et al., 2004). Separate pots were used for each date and site of collection. The pots were checked weekly for emerging adult gallers and parasitoids which were sorted to morphospecies, and stored in 2 ml plastic micro tubes in ethanol (or dry at -20°C in the case of micro Lepidoptera). Galler samples were identified by specialist taxonomists (V.C. Maia at Museu Nacional/UFRJ, Brazil and V.O. Becker at Reserva Serra Bonita, Brazil). Parasitoids were first sorted to family by MB, then identified to the lowest possible taxonomic level by M.T. Tavares, and are deposited in the Coleção Entomológica do Departamento de Ciências Biológicas at Federal University of Espírito Santo (UFES), Brazil. We calculated species richness and abundance of gallers and parasitoids, and parasitism rates as the proportion of individuals of each galler species successfully parasitized by different parasitoid species. Three gregarious parasitoid species from two different families emerged from three of the galler species (Family Eulophidae: *Horismenus* sp1, and *Tetrastichinae* sp1; family Trichogrammatidae: *Trichogrammatidae* sp1, Table S2 in Online Supplementary Information). They were present in 11.7% of samples of these three galler species and represented 66.3% of their reared parasitoids. Because several parasitoid individuals emerged from individual hosts, parasitism rates calculated on the basis of the ratio of parasitoid to host individuals would overestimate parasitism rates for samples containing gregarious parasitoids. Therefore, to avoid overestimation, we corrected the parasitism rates to take account of gregarious parasitoids, by subtracting the number of gallers emerged from the total number of galls in a sample. Then, based on the average number of each of the gregarious parasitoid species that emerged per gall across all samples we estimated the proportion of the remaining galls that were parasitized.

Food web metrics

We built matrices of interactions for each plot for each of the three sampling occasions. We calculated a suite of quantitative food web metrics, that when combined reflect the structure and robustness of the network (Tylianakis et al. 2007, van Veen et al. 2008, Kaartinen & Roslin 2012, Morris et al. 2014, Maunsell et al. 2014), to compare control and manipulated food webs:

(i) Weighted quantitative connectance - weighted ratio of realized trophic links to all possible links within the web (quantitative linkage density / number of species; Tylianakis et al. 2007);

(ii) Interaction evenness - Shannon's evenness of network interactions to measure uniformity of energy flows (Tylianakis et al. 2007);

(iii) Weighted quantitative linkage density - weighted diversity of interactions per species (average of vulnerability and generality; Tylianakis et al. 2007);

(iv) Robustness HL (robustness of the higher trophic level) - a measure of the tolerance of the network to node extinctions at the lower trophic level (in this case the tolerance of the food web to simulated extinctions of galler species; Burgos et al. 2007);

(v) Weighted quantitative vulnerability - average number of parasitoid species attacking each host, weighted by their marginal totals (Tylianakis et al. 2007);

(vi) Weighted quantitative generality - average number of host species attacked by each parasitoid species, weighted by their marginal totals (Tylianakis et al. 2007);

These quantitative metrics were calculated by weighting each interaction by its abundance following Bersier et al. (2002). Metrics were calculated in the statistical computing environment *R* (R Core Team, 2014) with the *bipartite* (version 2.04) package (Dormann et al. 2008, 2009) using the function networklevel with the argument empty.web set to false to account for hosts present but not parasitized (Morris et al. 2014). To investigate whether differences in metrics between treatments resulted solely from reduced abundance of *B. dracunculifoliae*, we compared webs between treatments with and without including *B. dracunculifoliae* in the calculation of metrics.

Statistical analysis

To investigate whether the response variables differed between manipulated and un-manipulated food webs we used the lme4 package (Bates et al. 2014) in R (R Core Team 2014). We compared parasitism rate, species richness, and abundance of both *B. dracunculifoliae* and other gallers between treatments by fitting a set of generalized linear mixed effect models (GLMMs), with Poisson errors for count data and binomial errors for proportion data (Crawley 2012). To minimise the chance of missing any effect of the treatment, we analysed all six response variables independently, even though some responses are inter-correlated. The structures of the maximal linear mixed effects models are shown in Appendix S1 in the Online Supplementary Information. We included treatment as a fixed effect in all our models; and when analysing galler abundance (non-focal species), parasitoid richness and parasitism rates we also included gall morphospecies as a fixed effect. We specified block as a random effect in all models. We checked the GLMM models for over-dispersion of residuals using the function overdisp.glmer (RVAideMemoire Package). To correct for over-dispersion where present, we refitted the models with negative binomial distribution using the function glmer.nb (MASS Package) instead of glmer. For species with sufficient replication we also tested for differences between treatments in abundance of individual species of parasitoids attacking other gallers.

For the metrics of food web structure, we fitted linear mixed effect models (LMMs) using treatment as fixed effect and month as covariate together with matrix size (the sum of all interactions between individuals recorded in a quantitative network matrix), as it can potentially bias quantitative metrics (Morris et al. 2014). We combined data from all months to increase our sample size. We used the testInteractions function (phia Package) to perform Wald chi-square test for *post hoc* comparisons between treatments across gall morphotypes. To determine the structure of the random effect in the models we compared models allowing for variation in intercept within random effect to those allowing for variation in intercept and slope, and selected models with lower Akaike Information Criteria (AIC) scores. We simplified maximal models by removing non-significant fixed effects to obtain a minimum adequate model (Crawley 2012). P-values of fixed effects were generated by likelihood-ratio tests of the full model with and without the explanatory variables. We then refitted the minimum adequate model using Restricted Maximum Likelihood (REML) and visually checked the residual plots for deviations from homoscedasticity or normality. Some of the web metrics were log-transformed to improve the homoscedasticity of residuals.

Results

Species richness, abundance and parasitism

Over the five-month study period we collected a total of 9,496 insect galls of 11 distinct morphospecies (Fig1; Table S1 in Online Supplementary Information), an average of 462 (SD = 165) galls from each control plot and 171 (SD = 63.7) from each exclusion plot. Galls induced by *B. dracunculifoliae* accounted for 83.1% of galls collected from control plots and 49.5% of those collected from exclusion plots. In total, 1,841 individual parasitoid wasps were successfully reared, representing 50 species (control = 40; exclusion = 33) within 16 families (Fig1; Table S2 in Online Supplementary Information). Gall removal significantly reduced the abundance of *B. dracunculifoliae* in treatment plots on average by 75.9% (SD = 16.9%) relative to corresponding control plots (Table 1). Parasitism rates of *B. dracunculifoliae* were significantly higher in plots where its abundance had been lowered (Table 1 and 2), whereas there was no significant difference in parasitism rates of the other gallers between treatments (Table 2). Species richness of parasitoids attacking *B. dracunculifoliae* did not differ significantly between treatments (Table 1). The *B. dracunculifoliae* removal treatment had no significant effect on abundance and richness of other gallers, but one galler species, *Asphondylia* sp2 (galler

G5 in Figure 1, Table S1 in Online Supplementary Information), was only found in control plots. Parasitoid richness of all gallers also did not differ between treatments (Table 1). However, the treatments differed considerably in parasitoid species composition, as 20 parasitoid species were only found in control plots, and 15 were only found in exclusion plots (Table S2 in Online Supplementary Information). We were able to test for differences between treatments in rates of parasitism on other gallers for three of the 50 species (*Bracon* sp2 attacking Tephritidae sp1, and *Tetrastichinae* sp3 and *Torymoides* sp2 attacking *Asphondylia* sp1; Table S1 and S2 in Online Supplementary Information). Of these, only parasitism by *Bracon* sp2 differed significantly between treatments, increasing by 98.3% in the manipulated plots (from 15.3% to 30.4%; Figure 1; Table S3 in Online Supplementary Information).

Food web metrics

All parasitoid species were specialised on a single gall species, except for three species with very low abundance (*Brasema* sp1, *Brasema* sp2, and *Aphelinus* sp.; Fig 1; Table S2 in Online Supplementary Information). As a result, generality equalled one for all plots and clearly did not differ between treatments; therefore, we did not test it statistically. The metrics connectance and robustness HL were significantly lower in the manipulated webs, while interaction evenness was significantly higher (Table 3). This might be expected as a direct effect of the reduction in abundance of *B. dracunculifoliae* and consequently in its parasitoids. However, significant differences in food web structure between treatments remained if these metrics were recalculated without including *B. dracunculifoliae*. In particular, connectance was significantly lower in manipulated webs, showing an interaction with matrix size (Table 3). Robustness HL was also significantly lower in the manipulated webs (Table 3). Interaction evenness showed contrasting patterns depending on whether *B. dracunculifoliae* is included in metric calculation: without including *B. dracunculifoliae* interaction evenness was significantly lower in manipulated webs in an interaction with matrix size and month (Table 3). There were no significant differences in linkage density and vulnerability between treatments whether or not *B. dracunculifoliae* was included in metric calculation (Table 3).

Discussion

The experimental reduction in abundance of the dominant galler species did not lead to evident secondary direct effects on its associated parasitoid species, but indirectly affected other galler species, as well as the structure and robustness of the food webs. In contrast to many previously published host-parasitoid food webs (e.g. Muller et al. 1999, Lewis et al. 2002) the experimental webs that we constructed were unusual in that virtually all parasitoid species were specialised on a single host and therefore there was virtually no potential for parasitoid-mediated indirect interactions (for example, apparent competition) between host species. Galler parasitoids and galler-parasitoid networks generally do not appear to be more specialised than other herbivore parasitoids (Hawkins 1994) or their respective networks (Morris et al 2014). However, the high host-specificity in our system is likely to explain why we did not find any effect of the experimental manipulation on parasitism rates, abundance or species richness of other galler species. Parasitism of the manipulated species was higher in the exclusion treatment, but the richness of parasitoid species attacking it did not differ significantly between treatments. The increased parasitism likely resulted from the adult parasitoids present during the manipulation having fewer hosts to attack, and therefore increasing attack rate on the hosts present. The duration of our experiment was five months, comprising multiple generations (up to 5) of both hosts and parasitoids, and therefore ample time was available for any long-term direct or indirect effects of the manipulation on other species to be revealed (Morris et al. 2004). However, experimental reduction of *B. dracunculifoliae* significantly changed the food web structure, in particular reducing structural complexity (indicated by reduced connectance and interaction evenness), and lowering robustness to species loss (measured as robustness HL). Although we reduced the abundance of *B. dracunculifoliae* in our experiment, rather than removing it entirely, we expected that for many processes such a drastic reduction in abundance would cause 'functional extinction' with similar effects to extinction (for example in our food web, potentially releasing other galler species from competition).

In the context of the highly specialised nature of the networks studied, to interpret our results we must understand which structural changes in the webs affected the metrics, and how the manipulation may have indirectly caused the structural changes. First, we discuss the experimental design and whether this may have contributed to the observed results. Although the control and treatment plots were sometimes in close proximity (10-50 metres apart), they were discrete and natural patches of host plants. Any gallers or parasitoids colonising a plot were subject to local ecological processes and conditions, therefore immigration from neighbouring plots seems unlikely to have influenced our results. Control plots could potentially have interfered with parasitoid searching behaviour, lowering the effect of reduced target galler abundance in exclusion plots. However, increased parasitism on the target galler in manipulated plots suggests that parasitoids stayed within their plot, parasitising a higher proportion of the remaining galls in their plot, rather than dispersing to other plots with higher gall abundance.

Changes in food web structure

The observed changes in web structure were ultimately due to changes in relative abundance and identity of parasitoid species, which affected the distribution of interaction frequencies without affecting the total parasitism rates of hosts. For instance, parasitism rate and abundance of the galler Tephritidae sp1 did not differ significantly between treatment and control plots, but there was a two-fold increase in the frequency of the parasitoid *Bracon* sp2 attacking it in the exclusion treatment. Generally, higher interaction diversity, resulting from more frequent interactions combined with greater evenness of interaction frequency distribution, will tend to increase connectance, interaction evenness, vulnerability, generality, and linkage density (Bersier et al. 2002, Tylianakis et al. 2007). Food web robustness is strongly influenced by connectance (Dunne et al. 2002) and will also be positively affected by interaction diversity. The reduction in interaction diversity observed in our treatment plots means that there was a lower number of interactions per species (connectance); a concentration of energy flow around fewer pairs of interacting species (interaction evenness); and that parasitoid species were more vulnerable to extinction (robustness HL). These observations concur with the idea that high interaction diversity in networks increases the rate of ecosystem processes and should stabilize them under fluctuating environmental conditions (Tylianakis et al. 2010).

How did the manipulation indirectly affect the interaction frequency distribution? Since there was essentially no potential for parasitoid mediated indirect interactions, these effects are unlikely to have propagated via trophic links documented in the webs. Food webs such as those documented in this study are only a subset of interactions isolated from a much more intertwined network of networks with direct and indirect trophic and non-trophic linkages (Pocock et al. 2012). Since our sampling focused on the galler-parasitoid community, we cannot clarify the exact mechanism through which food web structure was affected in our experiment. However, in the next section we suggest potential mechanistic explanations for our results drawn from our field observations and previous work.

Trophic and non-tropic indirect effects

Species and interactions that were not included in the documented food webs may also have been affected by the manipulation, and may provide the mechanism underlying the observed effects. These include natural enemies such as chewing insects (e.g., grasshoppers, caterpillars, and beetle larvae), which can eat part of the gall wall and lead to death of the galler nymph (Lara et al. 2002). Other candidates are ants, which can affect gallers negatively by predating them or interfering with their oviposition behaviour (Fagundes et al. 2005); or positively by lowering the abundance of free-feeding herbivores (Neves et al. 2011). Furthermore, since all the gallers share the same host plant species, there was also considerable potential for plant-mediated effects, although again these effects were not studied in this experiment. Plant-mediated effects could be trophically mediated (e.g., through resource competition between herbivores), or mediated via non-trophic links; for example, herbivores can interact indirectly by modifying the nutritional quality or induced defences of their shared host plant (Karban & Baldwin 1997, van Veen 2015). However, if such plant-mediated effects occurred, they did not influence the change in food web metrics across treatments through changes in the abundance and species richness of the gallers and parasitoids. Non-trophic indirect interactions can also be mediated via the physical environment. By manipulating defensive compounds of the host plant, galling insects can alter the chemistry of leaf-litter and lower rates of litter decomposition and nutrient release (Schweitzer et al 2005, Frost et al 2012, Künkler et al 2013). This could in turn, for instance, affect plant growth and quality, and therefore availability or suitability of oviposition sites for gallers.

More generally, the focal galler *B. dracunculifoliae* may have affected many other groups by acting as an ecosystem engineer (Jones et al. 1994, 1997, Odling-Smee et al. 1996, Bruno et al. 2003) - a species that modulates the availability of resources to other species by causing changes in biotic or abiotic materials, and thus modifies, maintains and/or creates habitats for other species. Both live and hatched galls serve as habitat for many other groups of so-called inquilines e.g., ants, aphids, spiders) that have the potential to interfere with gallers and parasitoids (Collevatti & Sperber 1997, Martinsen et al 2000, Ohgushi 2005, Crawford et al 2007), providing a possible mechanism for the observed results. The galls of *B. dracunculifoliae* remain attached to the plant after dehiscence, sometimes for many months, and gradually become dry and woody (Espírito-Santo & Fernandes 2002), thus creating a habitat for other species. These old hatched galls provide further possible routes through which the effect of the experimental manipulation could have spread through the web. Studies of other systems (e.g., Ohgushi et al. 2005) suggest that such habitat modification can have cascading consequences within food webs. Resolving which of these proposed mechanisms contribute to our results will require further study, and will necessitate further manipulative experiments beyond the work described here.

Conclusion

In conclusion, our study reveals that even in webs that appear poorly connected, perturbations may propagate via indirect links. Had our control webs been used in a computational model of species removal, no indirect secondary effect would have been detected since there were no connections among the galler-parasitoid subsets of the webs. In our field experiment, however, we have shown that it is possible for effects to spread through wider trophic and non-trophic links. These propagating impacts on food web structure following reduced species abundance have implications for conservation biologists. In particular, they highlight that changes in the abundance of even a single species can affect the tolerance of food webs to extinctions, and that there are many ways in which the impacts of species reduction or removal can spread through food webs, not all of which are predictable. The results here highlight the importance of experimental manipulation of food webs in the field to document trophic and non-trophic indirect interactions, and ultimately for better understanding the structure and dynamics of ecological communities (Fontaine et al. 2011, Kéfi et al. 2012). This poses new challenges for future studies, both empirical and theoretical. It would be greatly beneficial for our understanding of network structure if we were able to build more comprehensive networks, including direct and indirect trophic and non-trophic interactions across multiple trophic levels (Fontaine et al. 2011, Kéfi et al. 2012). Computational simulations that account for non-trophic indirect effects are also needed, but our results suggest that empirical data will be key to our understanding because of the numerous and often unpredictable opportunities for indirect effects via a variety of mechanisms and pathways.

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Author Contributions

MB and RM conceived and designed the experiment with contributions from OL and GW; MB collected the data; MB analysed the data with support from RM; all authors contributed to interpreting the results; MB wrote the first draft of the manuscript and RM led the editing. All authors contributed critically to the drafts and gave final approval for publication.

Data accessibility

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.5r2p2 (Barbosa et al. 2017).

Tables and Figures

Table 1. Comparison of control and treatment plots using likelihood ratio test for a variety of response variables. The values highlighted in bold are statistically significant ($P < 0.05$).

Response	**Explanatory variable**	**χ^2**	**d.f.**	**p**
Abundance of *B. dracunculifoliae*	Treatment	35.720	1	**<0.001**
Abundance of other galler species	Treatment : Gall morphotype	9.430	9	0.399
	Gall morphotype	160.000	9	**<0.001**
	Treatment	0.182	1	0.670
Parasitism (all gallers)	Treatment : Gall morphotype	20.409	10	**0.026**
Gall richness (all gallers)	Treatment	0.275	1	0.600
Parasitoid richness (all gallers)	Treatment : Gall morphotype	11.363	10	0.330
	Gall morphotype	158.080	10	**<0.001**
	Treatment	2.101	1	0.147

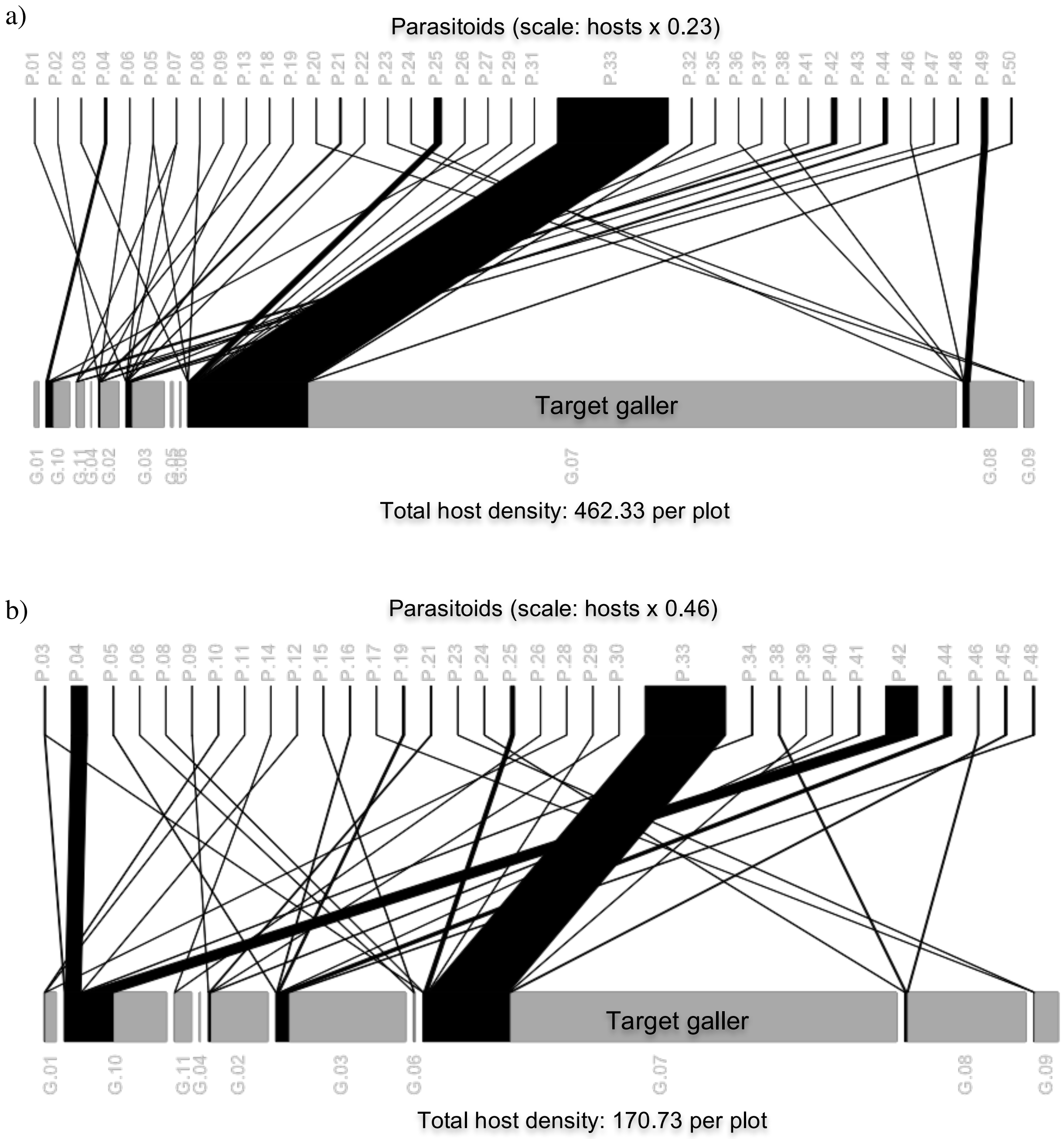
Table 2. Wald chi-square tests for *post hoc* comparisons of proportion of parasitism between treatments across gall morphotypes. G7 is the manipulated galler species. The value highlighted in bold is statistically significant ($P < 0.05$).

Variable	**Gall morphotype**	**Value**	**Df**	**Chisq**	**Pr(>Chisq)**
Parasitism	G1	0.000	1.000	0.000	1.000
	G10	0.481	1.000	2.345	1.000
	G11	2.750	1.000	0.960	1.000
	G12	0.668	1.000	0.000	1.000
	G2	1.612	1.000	0.525	1.000
	G3	1.312	1.000	0.313	1.000
	G5	1.021	1.000	0.000	1.000
	G6	0.000	1.000	0.000	1.000
	G7	3.380	1.000	10.186	**0.016**
	G8	3.548	1.000	5.411	0.200
	G9	1.524	1.000	0.183	1.000

Table 3. Comparison of food web metrics in control and manipulated plots using likelihood ratio tests. The values highlighted in bold are statistically significant ($P < 0.05$).

	With all galls				**Without *Baccharopelma dracunculifoliae***		
Response	**Explanatory variable**	**χ^2**	**d.f.**	**p**	**χ^2**	**d.f.**	**p**
Connectance (log)	Treatment : Month : Matrix size	1.11	2	0.575	0.91	2	0.636
	Month : Matrix size	0.21	2	0.900	0.07	2	0.966
	Treatment : Matrix size	0.22	1	0.643	5.48	1	**0.019**
	Treatment : Month	3.29	2	0.193	0.15	2	0.929
	Matrix size	5.12	1	**0.024**	-	-	**-**
	Month	10.62	2	**0.005**	7.48	2	**0.024**
	Treatment	5.83	1	**0.016**			
Interaction evenness	Treatment : Month : Matrix size	1.22	2	0.543	8.95	2	**0.011**
	Month : Matrix size	3.17	2	0.204			
	Treatment : Matrix size	0.00	1	0.984			
	Treatment : Month	7.24	2	**0.026**			
	Matrix size	13.86	1	**<0.001**			
Linkage Density	Treatment : Month : Matrix size	3.78	2	0.151	0.83	2	0.659
	Month : Matrix size	2.35	2	0.309	1.38	2	0.501
	Treatment : Matrix size	0.05	1	0.819	0.37	1	0.540
	Treatment : Month	1.96	2	0.376	0.38	2	0.826
	Matrix size	0.82	1	0.366	1.19	1	0.276
	Month	0.60	2	0.740	0.07	2	0.966
	Treatment	0.18	1	0.673	0.01	1	0.925
Robustness HL (higher trophic level)	Treatment : Month : Matrix size	1.41	2	0.494	0.58	2	0.748
	Month : Matrix size	3.31	2	0.191	0.28	2	0.869
	Treatment : Matrix size	0.28	1	0.598	0.54	1	0.463
	Treatment : Month	0.00	2	0.998	0.51	2	0.775
	Matrix size	0.86	1	0.355	8.85	1	**0.003**
	Month	21.01	2	**<0.001**	7.75	2	**0.021**
	Treatment	5.97	1	**0.015**	4.24	1	**0.039**
Vulnerability (log10 transformed for analyses without *Baccharopelma dracunculifoliae*)	Treatment : Month : Matrix size	3.78	2	0.151	0.68	2	0.711
	Month : Matrix size	2.35	2	0.309	1.35	2	0.510
	Treatment : Matrix size	0.05	1	0.819	0.63	1	0.428
	Treatment : Month	1.96	2	0.376	0.22	2	0.897
	Matrix size	0.82	1	0.366	1.23	1	0.268
	Month	0.60	2	0.740	0.39	2	0.823
	Treatment	0.18	1	0.673	0.07	1	0.789

Figure 1. Summary quantitative galler-parasitoid food webs, pooled across replicates, for (a) control and (b) manipulated (abundance of Baccharopelma dracunculifoliae experimentally reduced) plots. The upper bars represent parasitoid species and bottom bars represent galler species. Bar widths indicate the relative abundance of each species. The grey areas of lower bars indicate the proportion of gallers that were unparasitized from all galls sampled, whilst the black areas indicate parasitized gallers. The width of the links between the two trophic levels depicts the frequency of interactions. The species names of gallers and parasitoids corresponding to the abbreviations are listed in Table S1 and Table S2, respectively. To assist comparisons, the two webs are drawn at different scales. Total host density is shown beneath each network.



Appendix S1:

Model structures

1 - Abundance of other galler species between treatments

Random intercept model:

```
Model1 <- glmer.nb (gall_abundance ~ treatment : gall_morph + (1 | block))
```

Random intercept and slope model:

```
Model1 <- glmer.nb (gall_abundance ~ treatment : gall_morph + (treatment | block))
```

2 - Abundance of *B. dracunculifoliae* gallers between treatments

Random intercept model:

```
Model2 <- glmer.nb (gall_abundance ~ treatment + (1 | block))
```

Random intercept and slope model:

```
Model2 <- glmer.nb (gall_abundance ~ treatment + (1 + treatment | block))
```

3- Parasitism on galler species between treatments

Random intercept model:

```
Model3 <- glmer.nb (cbind (success, failure) ~ treatment : gall_morph + (1 | block))
```

Random intercept and slope model:

```
Model3 <- glmer.nb (cbind (success, failure) ~ treatment : gall_morph + (1 treatment | block))
```

4- Richness of other galler species between treatments

Random intercept model:

```
Model4 <- glmer (gall_richness ~ treatment + (1 | block), family = poisson)
```

Random intercept and slope model:

```
Model4 <- glmer (gall_richness ~ treatment + (1 + treatment | block), family = poisson)
```

5 - Richness of parasitoids on all galler species between treatments

Random intercept model:

```
Model5 <- glmer (parasit_richness ~ treatment : gall_morph + (1 | block), family = poisson)
```

Random intercept and slope model:

```
Model5 <- glmer (parasit_richness ~ treatment : gall_morph + (1 + treatment | block), family=poisson)
```

6 - Network metrics between treatments

Random intercept model:

```
Model6 <- lmer (metric ~ treatment * month * matrix_size (1 | block))
```

Random intercept and slope model:

```
Model6 <- lmer (metric ~ treatment * month * matrix_size (1+ treatment | block))
```

Table S1. List of morphospecies of galler insects collected on *Baccharis dracunculifolia* D. C. (Asteraceae). The abbreviations correspond to those used in Figure 1.

Abbrev.	**Galler insects**	**Order**	**Family**	**Organ**	**Shape**
G.01	Cecidomyiidae sp1	Diptera	Cecidomyiidae	Terminal bud	Rosette
G.02	Lepidoptera sp1	Lepidoptera	-	Stem	Fusiform
G.03	*Asphondylia* sp1	Diptera	Cecidomyiidae	Lateral bud	Globoid
G.04	Cecidomyiidae sp3	Diptera	Cecidomyiidae	Leaf	Globoid
G.05	*Asphondylia* sp2	Diptera	Cecidomyiidae	Terminal bud	Rosette
G.06	Lepidoptera sp2	Lepidoptera	-	Stem	Globoid
G.07	*Baccharopelma dracunculifoliae*	Hemiptera	Psyllidae	Leaf	Fusiform
G.08	*Geraldesia* sp1	Diptera	-	Leaf	Lenticular
G.09	Cecidomyiidae sp4	Diptera	Cecidomyiidae	Leaf and stem	Cylindrical
G.10	Tephritidae sp1	Diptera	Tephritidae	Terminal bud	Rosette
G.11	Cecidomyiidae sp5	Diptera	Cecidomyiidae	Stem	Globoid

Table S2. List of parasitoid wasps reared from each gall morphospecies collected in *Baccharis dracunculifolia* D. C. (Asteraceae) in control and exclusion treatments. The abbreviations correspond to those used in Figure 1. Asterisk (*) indicates gregarious parasitoid species.

Treatment	**Gall Morphospecies**	**Parasitoid Family**	**Parasitoid**	**Abrevv.**	**Specimens**
Control	G.02	Encyrtidae	*Anagyrus* sp2	P.02	1
		Eulophidae (Entedoninae)	*Horismenus* sp1*	P.21	16
		Eupelmidae	*Brasema* sp2	P.06	1
		Ichneumonidae	*Clydonium* sp1	P.09	1
			Scambus sp1	P.41	1
		Pteromalidae	*Pteromalidae* sp2	P.37	1
	G.03	Encyrtidae	*Anagyrus* sp1	P.01	1
			Psyllaephagus sp2	P.35	2
		Eulophidae	*Eulophidae* sp1	P.13	1
			Tetrastichinae sp3	P.44	38
		Eulophidae (Entedoninae)	*Horismenus* sp2	P.22	2
		Eupelmidae	*Brasema* sp1	P.05	2
			Brasema sp3	P.07	1
		Eurytomidae	*Eurytoma* sp2	P.19	3
		Torymidae (Megastigminae)	*Torymoides* sp2	P.48	11
	G.07	Aphelinidae	*Aphelinus* sp1	P.03	2
			Paraphytis haywardi (De Santis)	P.31	1
		Encyrtidae	*Metaphycus* sp1	P.26	2
			Psyllaephagus baccharidis	P.33	987
			Zaplatycerus sp1	P.50	12
		Eupelmidae	*Brasema* sp1	P.05	1
		Mymaridae	*Caraphractus* sp1	P.08	3

			Mymaridae sp2	P.29	1
		Platygastridae	*Platygastridae* sp1	P.32	7
		Pteromalidae	*Lyrcus* sp1	P.25	65
	G.08	Eulophidae	*Tetrastichinae* sp5	P.46	3
		Eulophidae (Entedoninae)	*Horismenus* sp4	P.24	2
		Eurytomidae	*Rileya* sp1	P.38	2
		Pteromalidae	*Pteromalidae* sp1	P.36	1
		Trichogrammatidae	*Trichogrammatidae* sp1*	P.49	55
	G.09	Eulophidae (Entedoninae)	*Horismenus* sp3	P.23	2
		Eurytomidae	*Eurytoma* sp3	P.20	2
	G.10	Braconidae (Braconinae)	*Bracon* sp2	P.04	21
		Encyrtidae	*Metaphycus* sp2	P.27	1
		Eulophidae	*Tetrastichinae* sp1*	P.42	49
	G.11	Eulophidae	*Tetrastichinae* sp2	P.43	1
		Eupelmidae	*Brasema* sp3	P.07	1
		Eurytomidae	*Eurytoma* sp1	P.18	2
		Torymidae (Megastigminae)	*Torymoides* sp1	P.47	2
Exclusion	G.01	Aphelinidae	*Encarsia* sp1	P.10	2
		Encyrtidae	*Encyrtidae* sp1	P.11	1
		Mymaridae	*Mymaridae* sp1	P.28	1
	G.02	Braconidae (Braconinae)	*Myosomatoides* sp1	P.30	1
		Eulophidae (Entedoninae)	*Horismenus* sp1*	P.21	4
		Ichneumonidae	*Clydonium* sp1	P.09	1
			Scambus sp1	P.41	5
	G.03	Eulophidae	*Tetrastichinae* sp3	P.44	27
		Eupelmidae	*Brasema* sp1	P.05	2
			Eupelmidae sp3	P.16	3
		Eurytomidae	*Eurytoma* sp2	P.19	7
			Rileya sp3	P.40	1

	Torymidae (Megastigminae)	*Torymoides* sp2	P.48	6
G.06	Eupelmidae	*Brasema* sp2	P.06	1
		Eupelmidae sp2	P.15	2
G.07	Aphelinidae	*Aphelinus* sp1	P.03	1
	Encyrtidae	*Psyllaephagus baccharidis*	P.33	265
	Eulophidae	*Tetrastichinae* sp4	P.45	7
	Eurytomidae	*Rileya* sp2	P.39	1
	Mymaridae	*Caraphractus* sp1	P.08	1
		Mymaridae sp2	P.29	1
	Pteromalidae	*Lyrcus* sp1	P.25	13
G.08	Eulophidae	*Tetrastichinae* sp5	P.46	4
	Eulophidae (Entedoninae)	*Horismenus* sp4	P.24	2
	Eurytomidae	*Rileya* sp1	P.38	5
G.09	Eulophidae (Entedoninae)	*Horismenus* sp3	P.23	2
	Eupelmidae	*Eupelmidae* sp4	P.17	1
G.10	Aphelinidae	*Aphelinus* sp1	P.03	3
	Braconidae (Braconinae)	*Bracon* sp2	P.04	52
	Encyrtidae	*Psyllaephagus* sp1	P.34	4
	Eulophidae	*Entedoninae* sp1	P.12	1
		Tetrastichinae sp1*	P.42	105
G.11	Encyrtidae	*Metaphycus* sp1	P.26	1
	Eupelmidae	*Eupelmidae* sp1	P.14	1
			Total	**1841**

Table S3. Regression coefficients from models of response variables on control and treatment groups. The values highlighted in bold are statistically significant ($p < 0.05$).

Response	**Coefficient**	**Estimate**	**s.e.**	**z**	**p**
Abundance of Bracon sp2 (P04)	Intercept	0.560	0.218	2.564	
	Treatment	0.827	0.259	3.197	**0.001**
Abundance of Tetrastichinae sp3 (P44)	Intercept	1.440	0.162	8.879	
	Treatment	0.064	0.252	0.253	0.800
Abundance of Torymoides sp2 (P48)	Intercept	0.201	0.302	0.666	0.506
	Treatment	-0.201	0.508	-0.395	0.693

CHAPTER 2

Submitted to Ecology

CHAPTER 2: An ecosystem engineer modifies interactions in a species-rich insect community

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Abstract

Theory suggests that non-trophic interactions are a major mechanism of community stability and persistence, but empirical data are scarce. Here we show that ecosystem engineering -physical changes to the biotic or abiotic environment caused by a species - can feed back to the engineer, modifying the magnitude and sign of its interactions with other species. Gall induction can be viewed as ecosystem engineering since both live and hatched galls serve as habitat for other species. In a field experiment, we generated treatments with reduced or elevated ecosystem engineering by removing or adding hatched galls. The manipulation had little effect on galler abundance and survivorship, but modified the galler-parasitoid and galler-aphid interactions. The effect of hatched galls on the interactions, and the interactions themselves, varied considerably in magnitude, and even sign, during the four month experiment. This work is one of the first to empirically demonstrate the regulatory role played by non-trophic interactions through interaction modification. The ubiquity of ecosystem engineering suggest that the effects found here are likely to be widespread and important. Future studies of community structuring must include non-trophic interactions as well as trophic interactions.

Key words: Aphids, Cerrado, experimental manipulation, galling insects, indirect interactions, interaction modification, parasitoid wasps, parasitism, rupestrian grasslands

Introduction

There is increasing evidence that at the local scale species interactions contribute considerably to the structure of ecological communities (Lawley et al. 2013). Historically, the main focus has been on direct and negative interactions, such as interference competition and predation (Menge & Sutherland 1976, Connell 1983). However, indirect interactions can also play a major role in community structuring (van Veen et al 2006, Morris et al 2004). Indirect trophic interactions, which require the presence of an intermediate third species (e.g. – trophic cascades), are likely to be responsible for many unexpected experimental results (Wootton 1994). In fact, many effects interpreted in the past as outcomes of competition may be a result of indirect trophic interactions, such as predator-mediated apparent competition (Holt 1977). Nevertheless, indirect interactions that are mediated non-trophically (e.g. – habitat creation/modification, facilitation) remain largely overlooked (Ohgushi 2008). Numerous shared physical conditions and resources can mediate interactions between species in non-trophic ways (Wootton 1994). It has been demonstrated empirically and theoretically that indirect non-trophic interactions, such as habitat modification, facilitation, and trait and behaviour modification (e.g., manipulation of plant chemistry by herbivores; predators striking fear into their prey), can play a role in community structuring and ecosystem functioning (Kéfi et al. 2012). As a result, neglecting non-trophic interactions (NTI) leaves out a vast amount of information on species interactions (Ohgushi 2008, Kéfi et al. 2015). It is unlikely that an understanding of community structuring and functioning can be achieved by solely focusing on specific trophic interactions (Kéfi et al. 2012, 2015).

One of the most common and important kinds of NTI seems to be ecosystem engineering (Kéfi et al. 2012, Sanders et al 2014), a process whereby a species, by causing a physical change to the environment (biotic or abiotic), modulates resource availability to other species (Jones et al. 1994, 1997). By definition, all organisms could be considered ecosystem engineers to some extent (Wright & Jones 2006). Since ecosystem engineering can change the environmental context it can impact not only the richness and density of species (node modulation), but also the interactions among them (link modulation; Sanders et al. 2014). However, the magnitude of engineering effect depends on engineer population density (Kéfi et al. 2012, Sanders et al. 2014, Wetzel et al. 2016) as well as on the densities of other species (Daborn et al. 1993, Jones et al. 1997). As a result, the engineering effect will change as a result of a variety of feedbacks that can alter engineer density and activity (Holt & Barfield, 2013, Sanders et al 2014).

The outcome of interspecific interactions can vary in sign (negative, neutral, or positive) and magnitude in response to biotic (e.g., species abundance) and abiotic (e.g., elevation) contexts (Chamberlain et al. 2014, Callaway et. al 2002, Menge et al 1994). This context dependence of interactions is a widespread phenomenon and possibly one of the main obstacles to understanding population and community dynamics in ecological and evolutionary time (Chamberlain et al. 2014). Therefore, there is a need to elucidate regulatory mechanisms underlying interaction variation in nature, rather than simply interaction outcomes. It is likely that NTI play an important role in the non-linearity of trophic interactions. However, the empirical studies that have investigated the effects of NTI at a community level (Hammill et al. 2015; Prasad & Snyder 2010; Prugh and Brashares 2012; Wetzel 2016), have mostly focused on changes in species density only.

Here we explored how NTI initiated by ecosystem engineering can feedback on the engineer, modulating its population density (node modulation) and affecting the engineer’s interactions with other species (link modulation). To investigate this process, we used a galler-parasitoid-aphid system based on the host plant Baccharis dracunculifolia D.C. (Asteraceae) in the Brazilian Cerrado. The focal galler, Baccharopelma dracunculifoliae (Sternorrhyncha: Psyllidae), is extremely abundant and gall induction can be viewed as ecosystem engineering (Wetzel et al. 2016). The galls remain attached to the plant for a few months after emergence of the galler, and gradually become dry and woody. Both hatched and unhatched galls of B. dracunculifoliae are occupied by many invertebrates, such as ants, spiders and aphids (Collevatti & Sperber 1997), which share the living space of the galler and are known as inquilines. The aphid Uroleucon tucumani (Sternorryncha: Aphididae) is by far the most frequent inquiline and its presence can lead to the death of galler larvae (Collevatti & Sperber 1997, Barbosa et al., unpublished).

We hypothesised that the hatched galls would mediate NTI, interfering with parasitism by wasps and inquilinism by aphids, and thus affecting the galler itself. Hatched galls could distract parasitoids from live galls increasing search times. Hatched galls could also have a positive effect on the galling insect by increasing the availability of shelter and therefore lowering the pressure of inquilines on live galls. Here, we assume a behavioural response where a fixed number of inquilines divide themselves among a number of occupied and unoccupied galls. However, it could equally be that in the longer term large inquiline populations build up where resources are abundant for them, generating the reverse effect. In a replicated field experiment, we excluded hatched galls from natural patches of the host plant and added them to other patches to test the effect of hatched galls on the galler, as well as on parasitism and inquilinism by aphids. Specifically, we tested:

(i) node modulation by hatched galls - whether hatched galls interfere with the abundance, survivorship, and volume (a measure of performance) of live galls;

(ii) link modulation by hatched galls - whether hatched galls affect the galler-parasitoid, galler-aphid, and parasitoid-aphid interactions. We expected changes in the relationship between the densities of each pair following manipulation of hatched galls.

Methods

Study Site

This study was conducted at Serra do Cipó, in Minas Gerais state, south-east Brazil. This region is in the southern portion of the Espinhaço Mountain Chain, in the Cerrado biome and is characterised by quartzitic soils covered by rocky grasslands, with a predominance of herbs and shrubs (Fernandes 2016). It has a Cwb Köppen climate type, with dry winters and rainy summers (Fernandes 2016). The average annual rainfall is between 1250 and 1550 mm, and the average temperature ranges from 18 to 19º C (Madeira & Fernandes 1999). The study sites were located alongside a 14 km section of the road MG-010 (from 19°17' - 19°15'S and 43°35' - 43°31'W). Altitude of the studied sites varied from 1028 to 1233m.

The study system

The host plant species B. dracunculifolia is a perennial, evergreen and dioecious shrub, 2-3m in height, which is widely distributed across the south central portion of South

America (Espírito-Santo et al. 2003). Populations of B. dracunculifolia frequently compose well-defined patches ranging from 18 to 12,000 m^2 (Collevatti & Sperber 1997). Among the 17 species of gall-inducing insects recorded on B. dracunculifolia, B. dracunculifoliae is the commonest (Fernandes et al. 1996) corresponding to approximately 83% of the galls (MB unpublished data). It induces a gall in the midrib of the leaf, which bends over itself until the borders are joined, modifying the entire leaf to form an elliptical, green, glabrous, single-chambered gall (Lara & Fernandes 1994).

Experimental design

We defined 30 plots, which were natural patches of approx. 15 to 20 plants of the plant B. dracunculifolia (hereafter "plots"), situated at least 20 m from each other and from other plants of the same species. The 30 plots were randomly assigned to ten blocks with three treatments each. In the plots assigned to the exclusion treatment all hatched galls of B. dracunculifoliae were removed through direct collection. Observations were made every two weeks to exclude newly hatched galls. The hatched galls collected from the exclusion treatment were "transplanted" to plants in the addition treatment plot within the same block, by attaching an equal number (average number of hatched galls collected per plant) of them to the stem of each plant, where they are usually found, using silicone glue. Before transplantation, invertebrates found inside the "empty" galls were removed with the aid of a slender brush. The other 10 plots served as controls. Non-galled leaves were removed from addition and control treatments in an equal number to that of hatched galls collected in the corresponding exclusion treatment plot. Also, silicone glue was applied to plants in exclusion and control treatment in an equal quantity and position to that used in the addition treatment plots.

Monitoring and data collection

The treatments were applied in July, one month before data collection. During the study period (August - October 2013) gall abundance was determined twice in each plot, in August and October. We counted all full-sized or close to full-sized galls of *B. dracunculifoliae* found in five half-metre branches haphazardly chosen around the crown of each of five individuals of *B. dracunculifolia* within each plot. The individuals sampled in August were marked and were not re-sampled in October. All live galls found on the chosen branches were collected in each plot. The live galls were reared for the emergence of adult gallers, parasitoids, and aphids by placing the galls in 250 ml plastic pots covered with nylon mesh (0.04mm net, 80% light penetration) and a perforated plastic lid (Espírito-Santo et al., 2004). Separate pots were used for each date and site of collection. The pots were checked weekly for emerging adult gallers and parasitoids which were sorted to morphospecies, and stored in 2 ml plastic micro tubes in ethanol. Parasitoids were first sorted to family by MB, then identified to the lowest possible taxonomic level by M.T. Tavares, and are deposited in the Coleção Entomológica do Departamento de Ciências Biológicas at Federal University of Espírito Santo (UFES), Brazil.

In addition, we measured gall size as an indicator of performance. We measured the width and length of galls to calculate their volumes according to their shapes (Volume $= 4/3\pi$ [1/2 Length] [1/2Width]^2, for ellipsoid galls), as in Dunham (1983). Data on gall abundance, performance and survivorship (proportion of gall from which adult gallers emerged), rates of parasitism, and density of aphids in live galls (aphids/gall) were initially aggregated for the two sampling periods to increase our sample size. However, when there was a significant interaction between a continuous variable and month, we analysed months separately to clarify the interaction.

Statistical analyses

To compare gall abundance and parasitoid richness across treatments we fitted generalised linear mixed effect models (GLMMs) with Poisson errors (Crawley 2013) using the lme4 package (Bates et al. 2014) in R (R Core Team 2014). We entered treatment as a fixed effect and month as a covariate. We included block as a random effect in both models, and also gall abundance in the model comparing parasitoid richness among treatments. We checked the GLMMs models for over-dispersion of residuals using the function overdisp.glmer (RVAideMemoire Package). To correct for over-dispersion we refitted the models with negative binomial distribution using the function glmer.nb (MASS Package) instead of glmer (ver Hoef & Boveng 2007, Bates et al. 2014). To determine the structure of the random effect in the models we compared models allowing for variation in intercept within random effect to those allowing for variation in intercept and slope and selected those with lower Akaike Information Criteria (AIC) scores. We performed simplifications of the maximal models by removing non-significant fixed effects to obtain a minimum adequate model (Crawley 2013). P-values of fixed effects were generated by likelihood-ratio tests of the full model with and without the explanatory variables. We then refitted the minimum adequate model using Restricted Maximum

Likelihood (REML) and visually checked the residual plots for deviations from homoscedasticity or normality.

For the analyses of effects on links between species (link modulation) we looked for variation among treatments in the slope of the relationship between pairs of groups – e.g., how parasitism varied with gall abundance in each treatment. We fitted general linear models for each response variable (parasitism, galler survivorship, and inquilinism) with either gall abundance or aphid density (depending on the model) in a three-way interaction with treatment and month, and plot as a covariate without interaction. For gall volume the model included only treatment and month as explanatory variables. Minimum adequate models were obtained, and models were checked, as described above. All dependent variables and gall abundance were log-transformed to improve the homoscedasticity of residuals, except for parasitism rate which was arcsine square-root transformed. Since the response variables were independent from each other we did not correct for multiple testing.

Results

In the exclusion treatment, we collected an average of 24.13 (SD = 8.57) hatched galls per plant at the beginning of the experiment, 32.46 (SD = 8.69) in August, and 29.79 (SD = 9.72) in October. Over the study period, we collected a total of 6,536 live galls induced by B. dracunculifoliae. The average abundance of live galls per plot was more than three times higher in August (M = 170.2, SD = 70.2) than in October (M = 53.3, SD = 24.6). In total, we successfully reared 2,144 individual parasitoid wasps, representing 15 morphospecies (exclusion = 7; addition = 9; control = 10) within six families.

Effects on species (node modulation)

There was no statistically significant difference in abundance of live galls or parasitoid richness among treatments (Table 1; Figures S1 and S2 Appendix). Gall abundance, but not parasitoid richness, was significantly higher in August than October (Table 1; Figures S1 and S2 Appendix). Galler survivorship (the proportion of adult gallers emerging from galls) was also not significantly affected by treatments (Table 1), but was significantly higher in August than in October (Table 1; Figure S3 Appendix). In October, gall volume was slightly greater in control than exclusion (Table 1; t= 3.587, df = 2, p = 0.004) and addition (Table 1; t = 4.151, df = 2, p<0.001).

Effects on interactions (link modulation)

Parasitism increased with gall abundance but the relationship varied between months (Table 1; Figure S4 Appendix). In August, parasitism was positively correlated with gall abundance with no difference among treatments (Table 1, Figure 1a); whilst in October the correlation was negative but varied among treatments (Table 1, Figure 1b). In October, the degree of density dependence of parasitism differed significantly between the addition and control treatments (t = 2.595, df = 1, p = 0.016), and also between the addition and exclusion treatments (t = 2.429, df = 1, p = 0.023), but not between the control and exclusion treatments (t = 0.303, df = 1, p = 0.765; Figure 1b). In addition, we found that parasitism was negatively correlated with aphid inquilinism overall with no difference among treatments (Table 1; Figure 2a), but the slope of the relationship was more negative in August than in October (Table 1; Figure 2b; t = 2.680; p = 0.010). There was also a significant positive correlation between galler survivorship and gall abundance (Table 1; Figure 3a), although the correlation did not differ among treatments (Table 1; Figure 3a) or between months (Table 1; Figure 3b).

Inquilinism was negatively correlated with gall abundance but the with difference in slope among treatments (Table 1; Figure 4) - the relationship for the control was significantly more negative than for the addition and exclusion treatments (t = 2.082, df = 1, p < 0.046; t=2.296, df = 1, p<0.029), but there was no difference in the slopes of addition and exclusion (t=0.091, df = 1, p<0.928; Figure 4). Aphid inquilinism was also higher in October than in August (Table 1, Figure S5 Appendix).

Discussion

In our community, we found evidence that an ecosystem engineering process (here the induction of galls) can feedback to the engineer and modulate its interaction with other species (Figure 5). Although some studies have demonstrated the effects of NTI at a community level (Hammill et al. 2015; Prasad & Snyder 2010; Prugh and Brashares 2012; Wetzel 2016), most of them have focused on changes in species density only. This is one of the first studies to provide empirical evidence for the role played by NTI through interaction modification. The manipulation of hatched galls had little effect on the galler (node modulation) - galler abundance, and survivorship were not affected, and gall volume changed only slightly - but modified the galler-parasitoid and galler-aphid interactions (link modulation; Figures 1b and 4 respectively).

Similarly to Wetzel et al. (2016), our study demonstrated how ecosystem engineering can shape community structure. In their oak tree - galler system, experimentally removing old, woody hatched galls influenced the structure of the arthropod community on foliage, through increasing herbivore density and richness, and reducing beta diversity. As a mechanism for the propagation of the effect, the authors suggest that the old hatched galls provided habitat for jumping spiders, which suppressed herbivorous arthropods. Here we move beyond a simple demonstration of NTI effects through changes in density of other species to reveal the role of NTI in interaction modification, as well as in initiating feedbacks, and consequently playing a key role in community regulation. The effects of the manipulation and the interactions were not consistent, and varied considerably in magnitude and even direction between months. NTI triggered by hatched galls therefore appear to change with abiotic and biotic conditions, such as the abundances of other species, or due to overlap with other NTI. We discuss these effects and their implications for community stability in detail below.

Interference among NTI

If indirect effects within a network feedback on each other and change interactions, the resulting effect of one species on another will be the product of several direct and indirect effects reinforcing or cancelling each other out. Total net effects will rarely be linear or direct, and are more likely to be diffuse and change with time as population densities fluctuate (Menge et al. 1994, Chamberlain et al. 2014, Figure S6 Appendix). For instance, Sanders and van Veen (2011) describe two opposing effects of ants in a grassland food web: by building mounds, ants increase primary productivity and therefore the densities of decomposers, herbivores and parasitoids; this engineering effect is however counteracted by the trophic effect of ant predation on herbivores and parasitoids. Therefore, experimentally manipulating one group may affect a specific interaction without affecting population densities, if other indirect effects are simultaneously counteracting the effect (Pintor and Soluk 2006). This suggests that ecosystem engineering may have a much more profound influence on community structure, which will only be detected if we investigate interaction modifications.

In the present study, we identified a number of opposing effects that could potentially overlap and amplify or nullify each other. For example, even though parasitism, in general, showed positive density dependence, galler survivorship was also positively correlated with gall abundance (Figure 6). This may be explained by lower density of aphid inside galls when gall abundance is higher. Similarly, at lower gall abundances, saturation of galls occupied by aphids would detrimentally affect parasitism rates. The negative correlation between parasitism rate and aphid density (Figure 2) indeed suggests that aphids could lower parasitism rate by killing the parasitised larvae. This reinforces the idea that hatched galls may have a significant positive impact on parasitism rates depending on whether hatched galls reduce or increase the occupation of live galls by inquilines. However, we were not able to assess the degree to which this happened, as collecting hatched galls would have interfered with the experimental treatments. On the other hand, parasitoids facilitated aphid colonisation of galls by making exit holes through which aphids can enter (MB personal observation). In summary, at different moments in time, as species densities vary, different combinations of overlapping effects will modulate interactions leading to non-linear dynamics (Figure 6).

Density-dependent feedbacks on NTI

Ecosystem engineering in this experiment modified interactions non-linearly and dynamically (differing in strength and direction with time). Besides being offset or reinforced by each other, NTI may also vary due to density-dependent feedbacks (Jones et al 1997, Sanders and van Veen 2011). NTI could introduce non-linearity in the relationship between species if the effect magnitude is regulated by feedbacks that depend on the density of the species involved (Figure S6). For instance, both addition and exclusion treatments showed less intense density-dependence of aphid density to gall abundance than the control (Figure 4), suggesting a non-linear response of aphids to the abundance of hatched galls - somehow both having too high or too low abundance of hatched galls had the same effect on gall occupation by aphids. Similarly, the changes in density dependence of parasitism and inquilinism with high and low gall abundance (Figure 6) may, in fact, be a result of modulation by density-dependent NTI. Parasitism, for instance, presented a u-shaped relationship to gall abundance - negative density dependence for lower gall abundance and positive for higher gall abundance (Figures 1 and 6). This may be a result of changes in parasitoid searching behaviour, modulated by changes in abundance of hatched galls. The density dependence of NTI may be the key mechanism behind many reported changes in interaction sign and magnitude (Chamberlain et al. 2014) and could explain in our experiment why the effects of manipulating hatched galls were not consistent but varied in magnitude and even direction between months.

NTI and stability

In our experiment the manipulation influenced the links, changing the interactions magnitude and sign, whilst population densities remained stable. These results suggest that due to the malleable nature of NTI, they could play a key role in stabilising food webs (see van Veen et al 2005). Providing further evidence for this idea, Hammill et al (2015) recently demonstrated that NTI can increase long-term persistence in a multi-trophic aquatic system through the weakening of trophic interactions by non-prey species. Their results highlight that persistence in complex food webs cannot be predicted from the dynamics of simplified systems, and that species not directly involved in trophic interactions are likely to play key roles in maintaining persistence.

In a previous study in our system, the exclusion of live gallers of the same abundant galler species from a galler-parasitoid food web reduced web complexity and robustness (chapter 1). These effects could not have propagated via the documented trophic links since the parasitoids were almost entirely specialised on single hosts and therefore there was virtually no potential for parasitoid-mediated indirect interactions. The results here illustrate the numerous ways by which NTI can mediate changes in the food web following species removal, and highlight the importance of considering indirect non-trophic links to improve our understanding of community structuring and stability (Ohgushi 2008, Kéfi et al. 2012, 2015).

The fact that a product of ecosystem engineering, such as a hatched gall, can play a role in interaction regulation suggests that indirect effects may be far more widely spread and influential than is realised. Every species has the potential to get involved in NTI, whether via engineering or other countless means (e.g., facilitation, interference competition, trait and behaviour modification), therefore it is very likely that a complex network of NTI is constantly modifying interactions and shaping ecological communities (Ogushi 2008). These concepts have been explored in theories such as niche construction - the process in which an organism alters its own or other species' niche (Odling-Smee et al. 2003), ecosystem engineering (Jones et al. 1994, 1997), and facilitation (Bruno et al. 2003) but have yet to be appropriately integrated into studies of community structure and dynamics (Kéfi et al. 2012, Sanders et al. 2014). Our results provide the first empirical evidence to show why this is essential.

ACKNOWLEDGEMENTS

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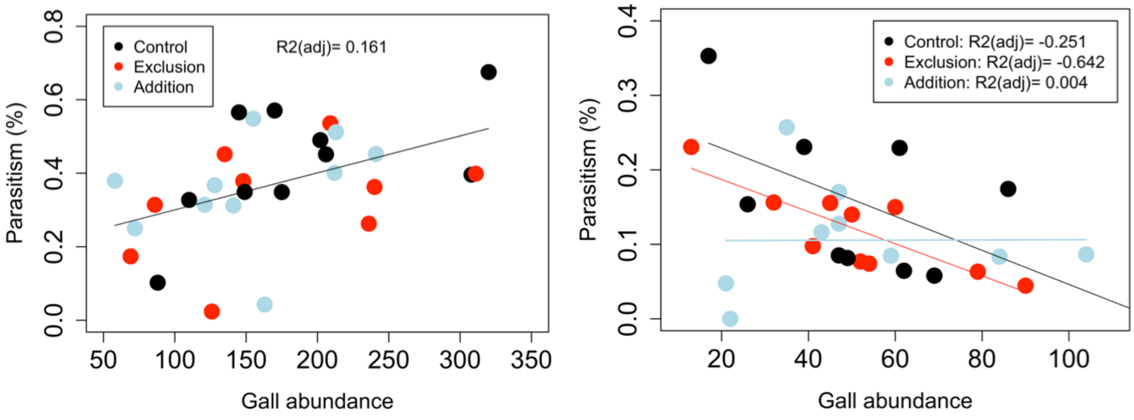
Tables

Table 1. Summary results of tests of Generalised Linear Mixed Models (glmm) and Linear Models (lm) for all variables tested. The values highlighted in bold are statistically significant ($P < 0.05$). P values were generated by likelihood-ratio tests of the full model with and without the explanatory variables. Only the explanatory variables of interest are reported.

Response	Explanatory variable	Result of statistical test
Gall abundance	Treatment : Month	glmm: $\chi^2(2) = 0.560$, $p = 0.756$
	Treatment	glmm: $\chi^2(2) = 2.589$, $p = 0.274$
	Month	glmm: $\chi^2(1) = 80.652$, $p <$ **0.001**
Parasitoid richness	Treatment : Month	glmm: $\chi^2(2) = 0.045$, $p = 0.977$
	Treatment	glmm: $\chi^2(2) = 0.792$, $p = 0.063$
	Month	glmm: $\chi^2(2)= 0.620$, $p = 0.431$
Gall volume	Treatment : Month	lm: R^2(adj)= 0.097; $F_{2,9} = 3.259$, $p <$ **0.001**
Parasitism	Gall abundance : month	lm: R^2(adj)= 0.536, $F_{1,9} = 8.737$, $p =$ **0.005**
	Gall abundance : Treatment (August)	lm: $F_{1,9} = 0.687$ $p = 0.513$
	Gall abundance (August)	lm: R^2(adj)= 0.162, $F_{1,9} = 6.406$, $p =$ **0.018**
	Gall abundance : Treatment (October)	lm: R^2(adj)= - 0.229, $F_{2,9} = 6.290$, $p =$ **0.007**
	Inquilinism : Treatment	lm: $F_{2,9} = 0.301$, $p = 0.742$
	Inquilinism : Month	lm: R^2(adj)= -0.650 , $F_{1,9} = 7.182$, $p =$ **0.010**
Galler survivorship	Gall abundance : Treatment	lm: $F_{2,9} = 0.825$, $p = 0.447$
	Gall abundance : month	lm: $F_{1,9} = 2.874$, $p = 0.097$
	Treatment	lm: $F_{2,9} = 0.056$, $p = 0.945$
	Gall abundance	lm: R^2(adj)= 0.568 , $F_{1,9} = 4.215$, $p =$ **0.045**
	Month	lm: R^2(adj)= 0.540, $F_{1,9} = 13.351$, $p <$ **0.001**
Inquilinism	Gall abundance : Treatment	lm: adjusted $R^2= 0.444$, $F_{2,9} = 3.378$, $p =$ **0.047**
	Month	lm: adjusted $R^2= 0.231$, $F_{1,9} = 4.895$, $p =$ **0.034**

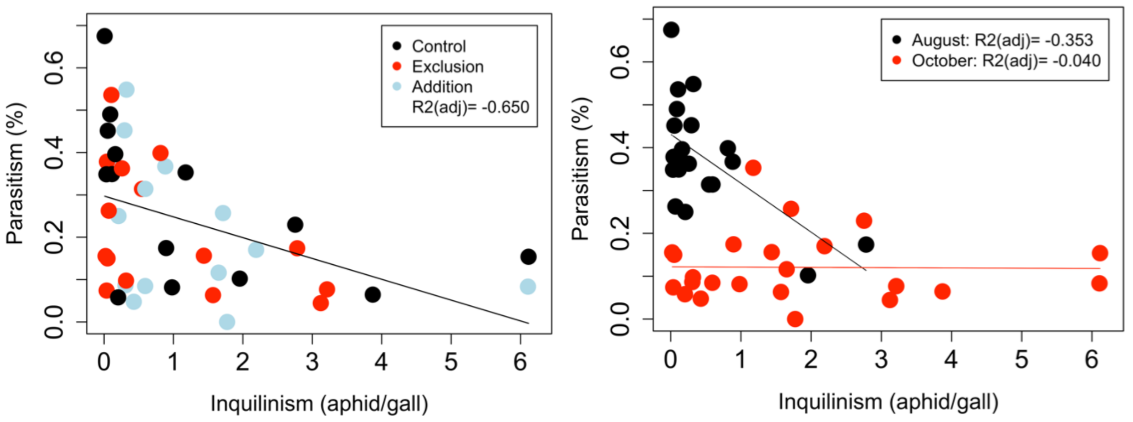
Figures

Figure 1. Relationship between parasitism rate and abundance of galls of *Baccharopelma dracunculifolia* in distinct treatments during August (a) and October (b). Solid lines represent fitted Linear Models


a) August

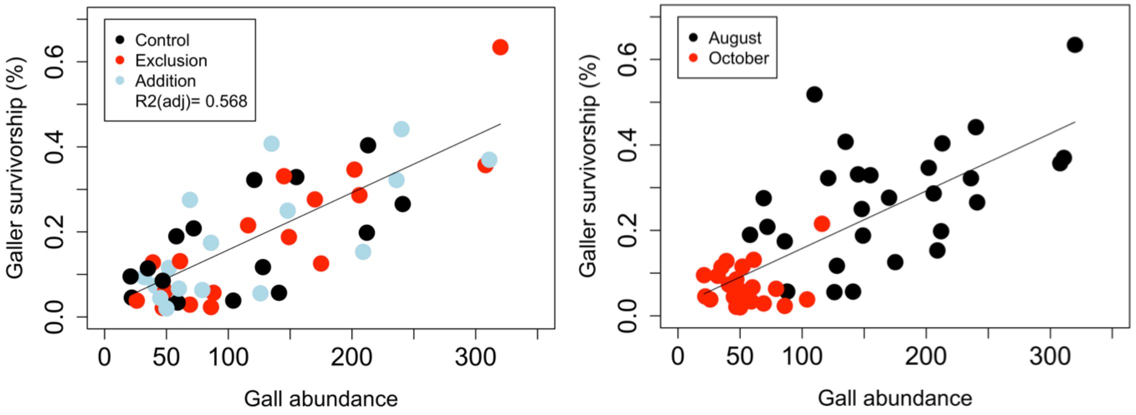
b) October

Figure 2. Relationship between parasitism rate of the galler *Baccharopelma dracunculifolia* and density of inquiline aphid by treatments (a) and by month (b). Solid lines represent fitted Linear Models


a)

b)

Figure 3. Relationship between survivorship and abundance of the galler *Baccharopelma dracunculifolia* by treatments (a) and by month (b). Solid lines represent fitted Linear Models


a)

b)

Figure 4. Relationship between density of inquiline aphid and abundance of galls of *Baccharopelma dracunculifolia* in distinct treatments. Solid lines represent fitted Linear Models

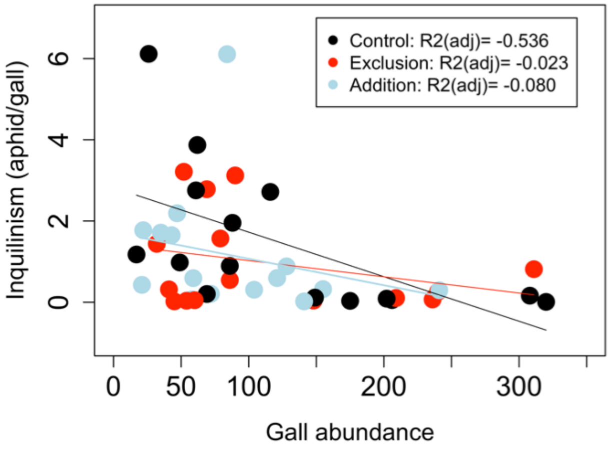


Figure 5. Interaction network based on field experiments on the host plant *Baccharis dracunculifolia* showing positive (+) and negative (-) effects categorised into two main types: node modulation (black arrows), which are pairwise trophic (solid arrows) and non-trophic interactions (dashed arrows); and link modulation, which are three-way interactions (interaction modification; dashed red arrows)

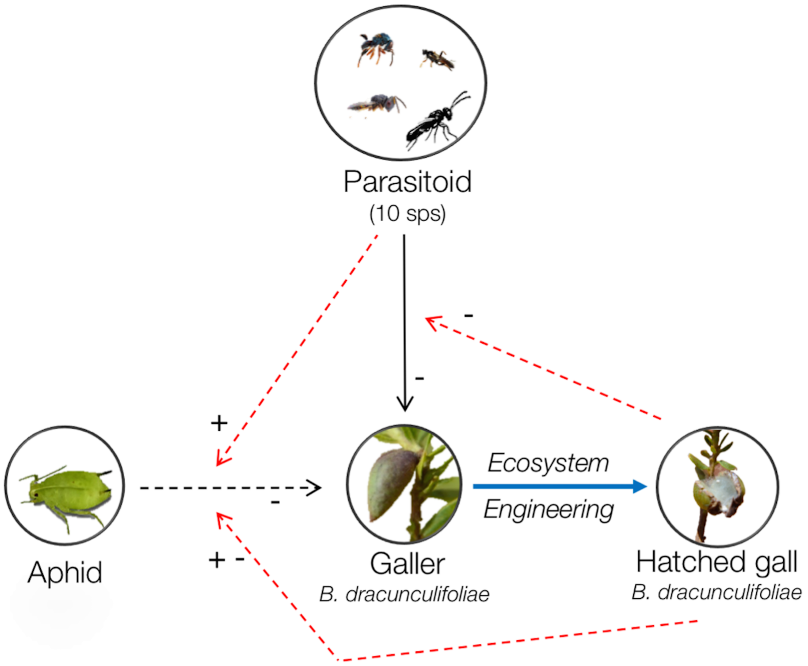
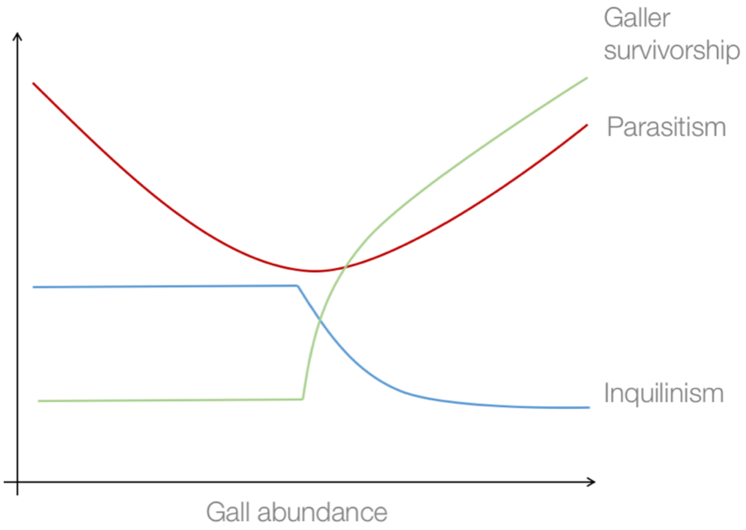


Figure 6. Sketch of putative non-linear variation of distinct processes in relation to abundance of galls of *Baccharopelma dracunculifolia* over time



Appendix

Supplementary material

Figure S1. Abundance of galls of *Baccharopelma dracunculifolia* by treatment and month

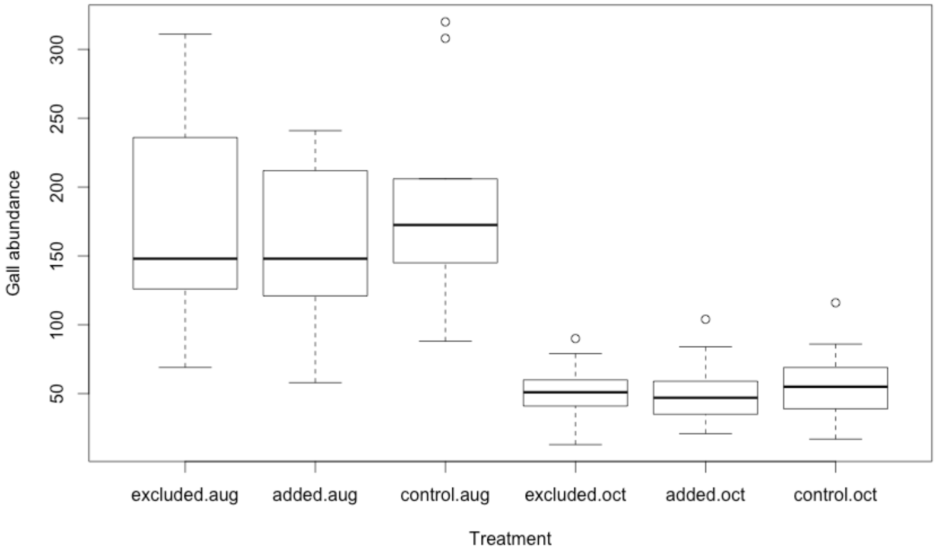


Figure S2. Species richness of parasitoids attacking the galler *Baccharopelma dracunculifolia* by treatment and month

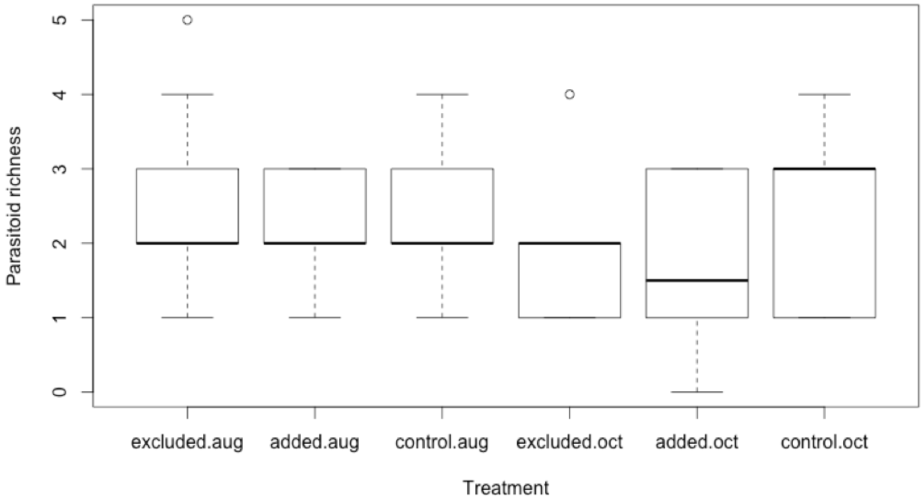


Figure S3. Survivorship rate of the galler *Baccharopelma dracunculifolia* by treatment and month

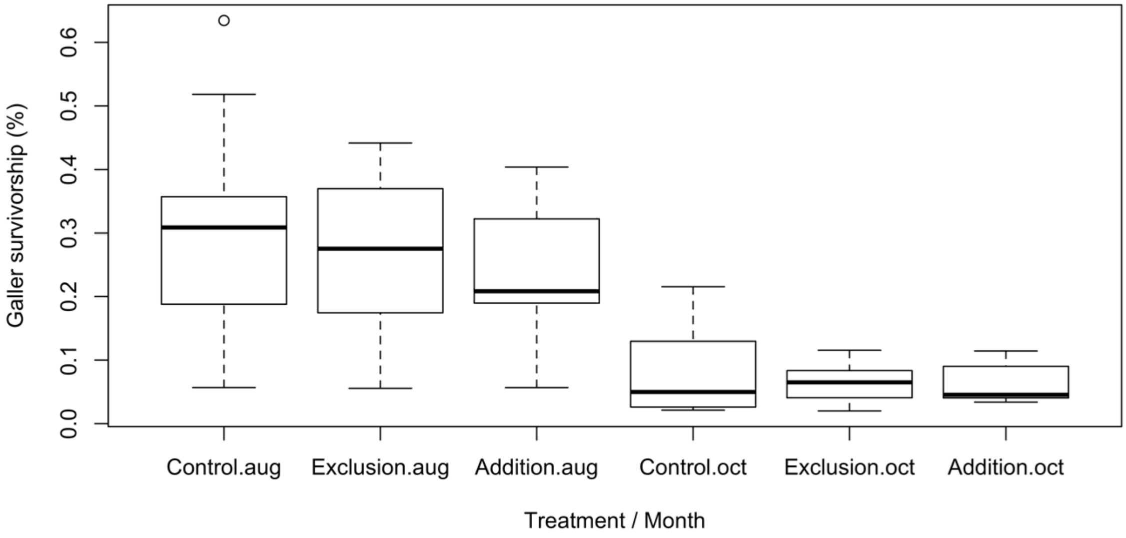


Figure S4. Relationship between parasitism rate and abundance of galls of *Baccharopelma dracunculifolia* in distinct treatments during August (a) and October (b). The solid line represents the fitted Linear Model

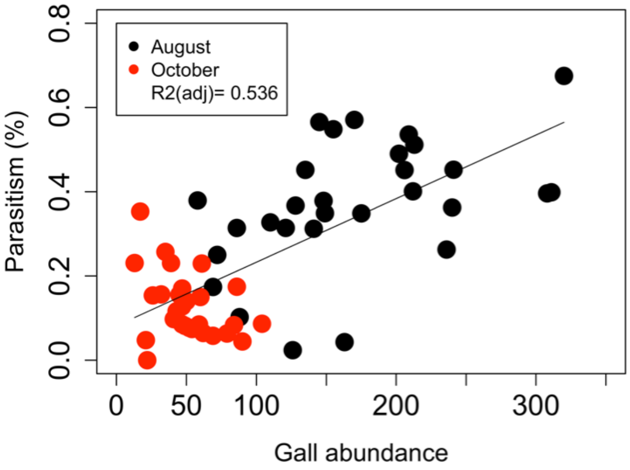


Figure S5. Density of inquiline aphids inside galls of *Baccharopelma dracunculifolia* by treatment and month

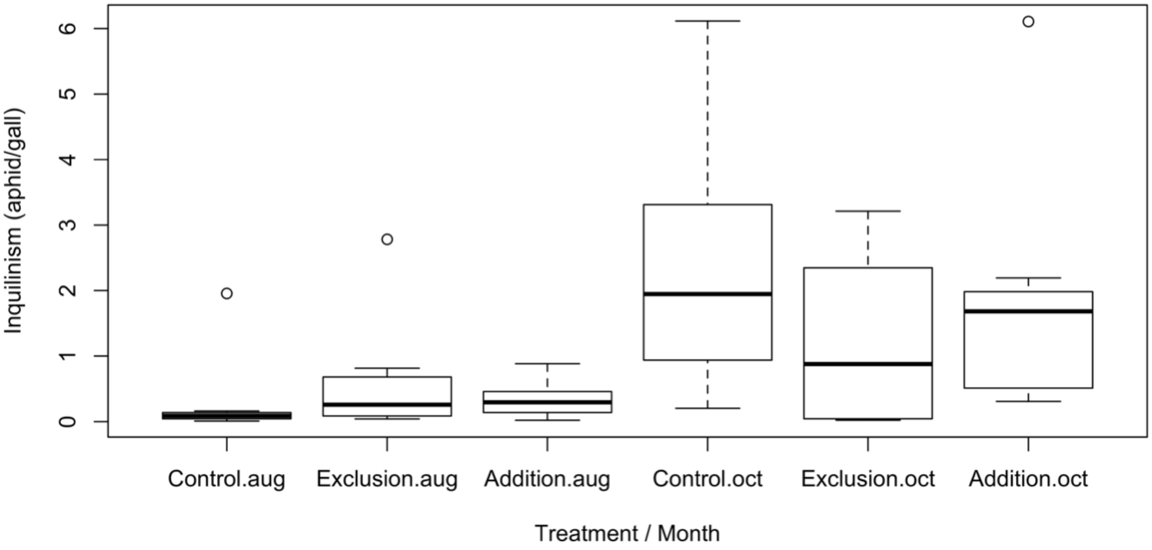
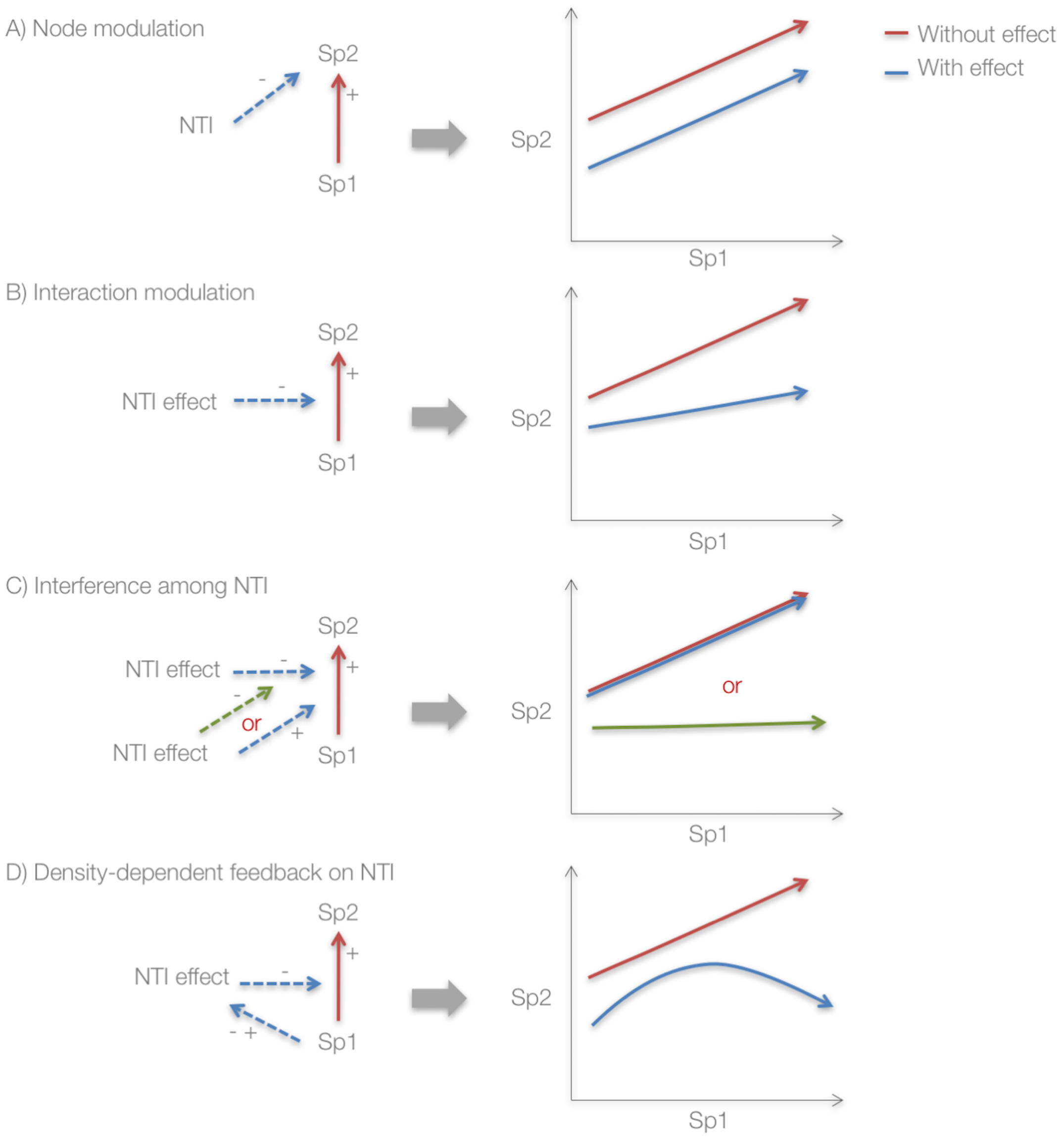


Figure S6. Diagram illustrating the expected outcomes of: (a) the effect of non-trophic interactions (NTI) on nodes, where only species density is affected, and (b) on interactions, where the relationship between densities of two species is altered; (c) the interference among overlapping effect of NTI, amplifying or cancelling each other out; and (d) density-dependent feedbacks on NTI changing its magnitude



CHAPTER 3

CHAPTER 3: Exploring the mechanisms behind interacting non-trophic interactions in an insect-plant community

Abstract

Both empirical and theoretical studies suggest that interaction modifications, where interactions are influenced by other interactions, cannot be ignored in predicting community responses to perturbations. However, the role of non-trophic interactions in interaction modification has rarely been tested empirically, particularly at the community level, and under field conditions. Here, I aimed to go beyond a simple demonstration of non-trophic interactions and to investigate the role of non-trophic interactions (NTI) in community structuring. Specifically, I tested mechanisms through which NTI could modify interactions and affect the structure and stability of a relatively simple and discrete ecological community in the field. I performed further manipulative experiments on a discrete arthropod community based on a shrub to test hypotheses on the mechanistic basis for NTI previously observed in this system. I investigated: (i) how reducing the abundance of the dominant galler indirectly affected food web structure and robustness (chapter 1), by looking at effects of reducing the galler abundance on other species not included in the food web; (ii) how aphids interfere with parasitism rate of the dominant galler (chapter 2), by testing the effect of aphid inqulinism on parasitoid mortality; (iii) how hatched galls interfere with parasitism rate of the dominant galler (chapter 2), by testing whether hatched galls lower inquilinism and, as a result, the negative effect of inquilinism on parasitism; (iv) whether hatched galls, aphid colonies, and ants tending aphids, interfere with abundance and parasitism of a second galler species (chapter 1). The results reveal that interaction modification by NTI is common among different sets

of species in this system, and is, therefore, likely to be widespread in nature. However, mechanistically explaining precisely how indirect interactions take place may be unfeasible even in relatively simple systems like this one. Different interactions combine to create net effects on population densities and are further evidence for how NTI can cement species together within a complex network.

Introduction

The factors structuring ecological communities and determining their stability have long intrigued ecologists (Hutchinson 1957, Chesson 2000). Species interactions are certainly important drivers of community structure, but large communities could also, in theory, be destabilised by random interactions (May 1972, McCann 2000). Thus, how are hundreds of species organised in highly complex and persistent communities (Lewis 2010, Andersen et al. 2012)? The traditional focus on trophic interactions in ecology overlooks the key role played by non-trophic interactions (NTI), which are mediated via numerous shared physical conditions and resources not involving feeding relationships (Wilson 1980, Wootton 1994) – e.g., habitat modification, facilitation, interference competition, ecosystem engineering, and trait and behaviour modification (Kéfi et al. 2012). As well as being important in their own right, NTI are likely to have a role in modifying trophic interactions and stabilising multi-trophic communities (Hammill et al. 2015). Further to trophic interactions, there has also been a focus in classical ecology on pairwise interactions. However, species are also involved in higher-order interactions, where a pairwise interaction is modulated by a third species, resulting in an indirect interaction (Wootton 1994, 2002). Such high-order interactions may involve trophic or non-trophic interactions and can play a role in weakening destabilising effects, such as a negative interaction between two species (Kelsic et al. 2015, Bairey et al. 2016). For instance, nonprey species may increase the persistence of predation-susceptible species by modifying the ability of predators to detect prey and altering the handling time and attack rate (Hammil et al. 2015). Likewise, consumer species may have a positive indirect effect on each other by reducing competition among their respective resource species (Sanders et al. 2015).

Ecological communities are not static objects and the fact that two species interact in one way in a given space and time does not mean that they will always interact in that same way (Havens 1992). Not only the local environmental conditions but also the community composition will influence the magnitude, sign and even the occurrence of interactions (Poisot et al. 2012, 2015). Both empirical and theoretical studies suggest that interaction modification, where interactions are influenced by other interactions, cannot be ignored in predicting community responses to perturbations (Fontaine et al. 2011, Kéfi et al. 2012, chapter 1). However, the role of NTI in interaction modification has rarely been experimentally tested, particularly at the community level, and under field conditions.

Here I aimed at going beyond the simple demonstration of NTI and explored possible mechanisms for interaction regulation by NTI in the field. I formulated and experimentally tested hypotheses on the mechanistic basis for NTI previously observed in a plant-galler-parasitid community (chapters 1 and 2). In chapter one, experimentally reducing the abundance of the commonest of 17 gall-making species associated with a host plant caused changes in structure and decreased robustness of the manipulated galler-parasitoid food webs. Parasitoid species were almost entirely specialised to individual galler species, so the observed effects of the manipulation could not have propagated via the documented trophic links. Instead, they must have spread either through trophic links not included in the webs (e.g., shared predators) or non-trophically (e.g., through changes in habitat availability). The authors hypothesised that the removal may have affected associated groups such as predators (e.g., ants, ladybirds, praying mantis, spiders) and free-feeding herbivores (e.g., aphids), or the host plant itself, which in turn may have interfered with the parasitoids and gallers via NTI.

Another study on the same system (chapter 2) demonstrated that hatched galls of the dominant galler, which serve as habitat for other species, can trigger NTI that will feedback to the galler modifying its interactions with other species. Chapter two also shows that the same dominant galler (galler G7 in chapter one), *Baccharopelma dracunculifoliae* (Homoptera: Psyllidae), has lower parasitism rates (measured from parasitoid rearing) when the occupation of galls by inquiline aphids is higher. Gall removal and consequent decrease in hatched galls could equally have interfered with the searching behaviour of parasitoids attacking other gallers, or changed the availability of habitat for predators and aphids. After removing the abundant galler, an identified change in parasitism of non-manipulated gallers was a two-fold increase in the frequency of a parasitoid species (Bracon sp2) attacking an apical galler, *Rachiptera limbata* Bigot (Diptera: Tephritidae; galler G10 in chapter 1). Thus, the possibilities of routes for NTI to spread through the system seem to be numerous. I performed further manipulative experiments on the same system to explore the mechanisms for NTI and specifically test whether:

1) Aphids interfere with parasitism rate of the galler *B. dracunculifoliae*:

    a. Colonising parasitized galls and killing the parasitized larvae. Parasitism would increase gall size (Espírito-Santo & Fernandes 2002) and aphids would be selective to larger galls, and therefore have a directed effect on parasitism rates;

    b. Attracting ants that interfere with parasitoids on branches.

2) Reducing the abundance of galls induced by *B. dracunculifoliae* affects the species richness, abundance, and interactions of aphids, ants, predators, and free-feeding

herbivores, as well as host plant growth. Changes in abundances of any of these groups, or in the interactions among them, following gall removal would give an indication of a potential pathway through which the effect of the manipulation could spread, affecting the entire food web structure.

3) Hatched galls interfere with parasitism of the galler *B. dracunculifoliae*:

    a. Increasing survival of parasitised galling larvae by reducing the colonisation of live galls by aphids;

    b. Increasing the abundance of aphids and ants that could interfere with parasitoids;

4) Hatched galls, aphid colonies, and ants tending aphids interfere with abundance and parasitism of the apical galler *R. limbata*. Since aphids occupy mainly the terminal buds and attract ants, it is likely that aphid interferes with the galler *R. limbata*. In addition, if hatched galls could interfere with aphids, they could indirectly affect the galler *R. limbata* as well.

By testing this set of hypotheses my aim was to go beyond purely demonstrating who affects who and how intensely, and to better understand the role of NTIs in community structuring. Specifically, I aimed to identify the mechanisms through which NTI can modify interactions and affect the structure and stability of a relatively simple and discrete ecological community.

Methods

Study Site

This study was conducted at Serra do Cipó, in Minas Gerais state, south-east Brazil. This region is in the southern portion of the Espinhaço Mountain Chain, in the Cerrado biome and is characterised by quartzitic soils covered by rocky grasslands, with a predominance of herbs and shrubs (Fernandes 2016). It has a Cwb Köppen climate type, with dry winters and rainy summers (Schulz & Machado 2000). The average annual rainfall is between 1250 and 1550 mm, and the average temperature ranges from 18 to 19ºC (Madeira & Fernandes 1999). The study site (19°16'48"S - 43°35'20"W; 1170 m elevation) is undergoing restoration with native species since 2010, after serving as a source of soil for the paving of the MG-010 highway. The plant species *Baccharis dracunculifolia* D.C. (Asteraceae) is one of the species planted and is now dominant in the area (see Fernandes et al. 2015).

The study system

The plant species *B. dracunculifolia* is a perennial, evergreen, dioecious shrub, 2-3 m in height, which is widely distributed across southern and central South America (Barroso 1976, Espírito-Santo et al. 2003). *B. dracunculifolia* frequently forms well-defined patches 18 to 12,000 m^2 in area (Collevatti & Sperber 1997). It has a key role in natural succession and regeneration (Fernandes et al. 2016) and is, therefore, important in terms of biodiversity and ecosystem functioning. The plant species hosts a diverse fauna of free-feeding herbivores, mostly Hemiptera, Coleoptera and Orthoptera, and also many predators including the orders Araneae, Coleoptera, Mantodea, and Hymenoptera. Among the 17 species of gall-inducing insects recorded on *B. dracunculifolia* in multiple locations (Fernandes et al. 1996, 2014), *Baccharopelma dracunculifoliae* (Sternorrhyncha: Psyllidae) is the commonest (Araújo et al. 1995. It induces a gall in the midrib of the leaf, which bends over itself until the borders are joined, forming an elliptical, green, glabrous, single-chambered gall that usually harbours up to four nymphs (Lara & Fernandes 1994). The gall is not completely sealed as the leaf borders are joined but not fused. The galls remain attached to the plant after dehiscence and gradually become dry and woody (Espírito-Santo & Fernandes 1998). Both hatched and non-hatched galls of *B. dracunculifoliae* are occupied by many inquiline invertebrates, such as ants, spiders, aphids, etc. (Mani 1964, Collevatti & Sperber 1997, Espírito Santo & Fernandes 2002). At least ten parasitoid species have been reared from galls of *B. dracunculifoliae* (chapter 1) and parasitism rates are around 45% (Espírito-Santo & Fernandes 2002). When the parasitoids emerge, they leave a characteristic exit hole on the gall wall (Espírito-Santo et al. 2004), facilitating aphid colonisation of parasitised galls (MB *pers. obs.*).

The aphid *Uroleucon tucumani* (Sternorryncha: Aphididae) is by far the most frequent inquiline (species that occupy a living space produced by another species – e.g., a gall) and can indirectly kill the nymphs of the gall maker (Collevatti & Sperber 1997, Fagundes et al. 2005, MB *pers. obs.*). This aphid species also feeds and reproduces on the apical meristems of the host plant, forming dense colonies that produce honeydew (sugary secretions). *U. tucumani* attracts at least 15 species of ants, which tend and protect them in a trophobiotic relationship (Fagundes et al. 2005, Neves et al. 2011, MB *pers. obs*). It has been found that the presence of ants reduces the number of *B. dracunculifoliae* nymphs per gall, and the presence of aphids on branches reduces *B. dracunculifoliae* gall size because aphids compete with the galler for sap assimilates and young leaves in terminal buds (Fagundes et al. 2005). Neves et al. (2011) observed that the presence of ants and aphids on *B. dracunculifolia* decreased the abundance of other free-feeding herbivores and that the presence of aphids decreased plant shoot growth. Ants tending aphids can have a direct negative impact on herbivores (Abe 1988, Fernandes et al. 1999). However, the aphids on their own can also reduce the abundance of fluid-sucking and chewing insects due to exploitation competition or by altering the nutritional quality of the host plant (Fay et al. 1996, Larson & Whitham 1997).

Experimental design

Sixty-four isolated individuals of the plant species *Baccharis dracunculifolia* D.C. (Asteraceae) of 1.5-2.0m in height, at least 5m distant from conspecific plant individuals but in the same area, were randomly identified and marked in the field. The plants were randomly assigned to four treatment groups (16 plants each) in blocks at least 20m apart from each other. Thus, there was one plant individual for each treatment in each block, and 16 blocks altogether. Different manipulations were performed in each treatment according to the hypothesis being addressed:

(i) *Ant Exclusion* - Ants were excluded by applying a non-toxic resin (Tanglefoot®, Tanglefoot Company, Michigan, USA) to the basal stem of the plants. During the study period, the plant individuals were monitored twice a week to check the effectiveness of the treatment, which was repeated where necessary;

(ii) *Live Gall Exclusion – B. dracunculifoliae,* the commonest galling species found on *B. dracunculifolia* was removed by direct collection. Since new galls could be induced over the monitoring period, the treatments were maintained by excluding newly induced galls every week, which prevented any galls from reaching full development;

(iii) *Hatched Gall Exclusion* - All hatched galls of *B. dracunculifoliae* were excluded from each of 16 plant individuals. Weekly observations were performed to exclude newly hatched galls;

(iv) *Control* - Non-galled leaves were removed in an equal amount to the average number of galls collected in treatments Live Gall Exclusion and Hatched Gall Exclusion. The treatment Ant Exclusion also had the same amount of non-galled leaves removed to allow comparison with control.

Monitoring and data collection

During the study period (August - September 2015) I combined direct observation and gall dissection data to quantify densities of several arthropod groups in each treatment as well as the frequency of direct interactions, including parasitism and inquilinism.

Observations – I performed weekly observations on the plant individuals during the study period. The species and abundance of arthropods on isolated plants were quantified by directly counting individuals and morphospecies for 10 minutes per week (between 9:00am and 3:00pm), totalling one hour per plant over the two months. I quantified the number of individuals of each species (or morphospecies) of ants, predators and free-feeding insect herbivores, as well as the number of branches occupied by ants (ant presence), and terminal buds occupied by aphids (aphid presence). During the study period, at least one individual of each morphospecies was collected for identification. Plant shoot growth was also measured using a tape measure for treatments Control and Live Gall Exclusion to test the effect of the galler on plant growth. On the final week of the eight-week study period, I quantified the abundances of live and hatched galls of *B. dracunculifoliae* (except in the treatments in which they had been excluded). I counted all full-sized or close to full-sized live galls and all hatched galls of *B. dracunculifoliae* found in three half-meter branches haphazardly chosen around the crown of each individual of *B. dracunculifolia*. I also quantified the abundance of the apical galler *R. limbata* on the same branches.

Gall dissection – After counting the aforementioned galls, I collected 15 of each gall type, *B. dracunculifoliae* and *R. limbata* (five of each type per branch). They were stored individually and taken to the lab for dissection. To calculate parasitism rates I dissected the galls and quantified the proportion of parasitised and non-parasitised nymphs per gall through the presence of "mummies" (parasitised galler nymphs). I also determined inquilinism rate (aphids per gall; not to be confused with "aphid presence" on branches) of the galler *B. dracunculifoliae*. Before dissection, galls were also measured for width and length to calculate their volumes according to their shapes (Volume = $4/3\pi$ [1/2 Length] $[1/2\text{Width}]^2$, for ellipsoid galls), as in Dunham (1983).

Hypothesis test

Hypothesis 1a - To test whether the aphids interfere with the parasitism of the galler by colonising parasitized galls and killing the parasitized larvae, first, I tested for a relationship between parasitism of the galler *B. dracunculifoliae* and inquilinism by the aphid. Then, I quantified the proportion of parasitised and non-parasitised galls that were occupied by aphids to see if aphids preferred parasitised galls, and compared the mortality of both parasitised and non-parasitised nymphs between galls with and without aphids. To determine whether parasitism increased gall volume I also compared gall volume between parasitised and non-parasitised galls. To verify whether aphids were selective to larger galls I examined the relationship between gall volume and inquilinism by the aphid.

Hypothesis 1b - In order to test whether the aphid influenced the parasitism rate of *B. dracunculifoliae* by attracting ants that interfere with the parasitoids, I tested for a relationship between parasitism rate of *B. dracunculifoliae* (from dissection data, described above) and frequency of both aphids and ants observed on branches (aphid, and ant presence), as well as ant richness. I also tested for a relationship between aphid presence and ant presence on branches. Since I assessed several ant species together, and some are smaller and more abundant than others, abundance would be a poor representation of ant activity. Therefore, I used the number of branches with ants as a measure of ant activity. I tested the effect of ant exclusion on aphid inquilinism, and parasitism of the galler *B. dracunculifoliae*; and also tested whether the relationship between parasitism and aphid inquilinism changed following ant exclusion, by comparing the slope of the relationship between Control and Ant Exclusion treatments.

Hypothesis 2 - To investigate whether reducing the abundance of the commonest galler affected the species richness, abundance, and interactions of other groups, I compared control and treatment Live Gall Exclusion in regard to the abundance and species (or morphospecies) richness of predators (e.g., ladybirds, praying mantis, spiders), free-feeding insect herbivores, ants and aphids (outside galls) determined through the observational method described above. Shoot growth was also compared between the Control and Live Gall Exclusion treatments.

Hypothesis 3a – To test whether hatched gall interfered with parasitism on the galler *B. dracunculifoliae* through reducing inquilinism of live galls I used data from the Control to investigate the relationship between the abundance of hatched galls and aphid inquilinism; and between the abundance of hatched galls and parasitism rate of *B. dracunculifoliae*. I also compared parasitism rate of the galler *B. dracunculifoliae* between the Control and the Hatched Gall Exclusion treatment.

Hypothesis 3b - to test whether hatched gall interfered with parasitism on the galler *B. dracunculifoliae* by attracting aphids and ants, I compared ant richness, ant presence, aphid presence, and inquilinism between the Control and Hatched Gall Exclusion treatment. I also tested for a relationship between abundance of hatched galls and ant presence as well as aphid presence on branches in treatment Control (evidently, the abundance of hatched galls could only be quantified for Control and Ant Exclusion treatments).

Hypothesis 4 - To investigate how hatched galls, aphid and ant presence on branches, interfered with the abundance and parasitism of the apical galler *R. limbata*, I tested for a relationship between the abundance and parasitism rate of the galler *R. limbata*, and the abundance of hatched galls, the presence of aphids, and the presence of ants on branches. I also compared abundance and parasitism rate of the galler *R. limbata* between the Control and treatment Live Gall Exclusion.

Statistical analyses

Observation – I used data from the weekly observations to compare treatments in terms of the species and abundance of ants, predators and free-feeding insect herbivores, as well as the number of branches occupied by ants (ant presence); and terminal buds occupied by aphids (aphid presence). In each case, I fitted either generalised linear mixed effect models (GLMMs; Crawley 2007) or, whenever it was not possible to obtain a satisfactory model fit, linear mixed effect models (LMMs) after square-root or log-transforming the data. I used the lme4 package (Bates et al. 2014) in R (R Core Team 2014) to fit the models. I entered treatment as a fixed effect, week as a covariate and plant individual as a random effect in all GLMM models. I used the testInteractions function (phia Package) to perform Wald chi-square test for *post hoc* comparisons between treatments. I also regressed ant presence against aphid presence entering treatment as a covariate with interaction and week and plant individual as a random effect in each model. I used the testInteractions function to perform the pairwise comparisons of adjusted slopes with respect to the response variable for contrasts of the factor treatment (Martínez 2013).

I checked the GLMMs models for over-dispersion of residuals using the function overdisp.glmer (RVAideMemoire Package). To correct for over-dispersion I refitted the models with negative binomial distribution using the function glmer.nb (MASS Package) instead of glmer. To determine the structure of the random effect in the models I compared models allowing for variation in intercept within random effect to those allowing for variation in intercept and slope, and selected the ones with lower Akaike Information Criteria (AIC) score. I performed simplifications of the maximal models by removing non-significant fixed effects to obtain a minimum adequate model (Crawley 2013). P-values of fixed effects were generated by likelihood-ratio tests of the full model with and without the explanatory variables. I then refitted the minimum adequate model using Restricted Maximum Likelihood (REML) and visually checked the residual plots for deviations from homoscedasticity or normality. No correction for multiple tests was done because the response variables were independent from each other.

Dissection - Using the data from gall dissection I tested for a relationship between inquilinism (aphid/gall) and parasitism; volume of *B. dracunculifoliae* galls and parasitism; volume of *B. dracunculifoliae* galls and inquilinism; entering treatment as a covariate in all models. For all analyses of dissection data, I used block as a random effect. Minimum adequate models were obtained, and models were checked, as described above.

Observation plus dissection – In order to be combined, the observation and dissection datasets had to be averaged across weeks and plant individuals, respectively. Therefore, it was not possible to use mixed-effect models, as there were not enough replicates to assign block as a random effect. I fitted general linear models (GLMs) with binomial distribution for each response variable (parasitism of gallers *B. dracunculifoliae* and *R. limbata*) against the explanatory variables (species richness and abundance of ants; ant presence; aphid presence; and abundance of hatched galls of *B. dracunculifoliae*) in an interaction with treatment. I also used GLMs to compare treatments regarding the abundance of *B. dracunculifoliae* and *R. limbata* galls and plant shoot growth. I used ordinary linear models to test the relationship between abundance of *R. limbata* galls and abundance of hatched gall of *B. dracunculifoliae*; inquilinism; ant richness and presence; as well as the relationship between inquilinism and abundance of hatched gall of *B. dracunculifoliae*. All models had treatment as a covariate with interaction. Minimum adequate models were obtained, and models were checked, as described above. When necessary, the depended variables were square-root or log-transformed to improve the homoscedasticity of residuals.

Results

Throughout the entire two-month observation period I quantified 1,427 ants from 15 different species found on 988 branches, 1,109 predators from more than 9 morphospecies (spiders, for instance, were grouped together as a single morphospecies), 629 free-feeding herbivore insects from more than 41 morphospecies, and 22,564 terminal buds occupied by aphids. I quantified 1,346 hatched galls, and 1,260 live galls of the galler *B. dracunculifoliae*, of which 522 live galls were collected and dissected. I also collected and dissected 92 live gall of the galler *R. limbata*.

Hypothesis 1a - ***Aphids interfere with parasitism of the galler B. dracunculifoliae by killing the parasitised larvae.***

Parasitism of the galler *B. dracunculifoliae* showed a significant positive relationship with inquilinism by aphids (Table 1, line 43; Figure 1). Inquilinism by aphid was significantly higher in parasitised galls, with no difference among treatments (Table 1, line 35; Figure

S1 in Appendix). Mortality of parasitised nymphs was significantly higher in galls occupied by aphids regardless of treatment (Table 1, line 55; Figure S2 in Appendix). Mortality of non-parasitised nymphs was significantly positively related to inquilinism by aphids regardless of treatment (Table 1, line 58; Figure S3 in Appendix). There was no significant relationship between gall volume and parasitism of *B. dracunculifoliae* (Table 1, line 48). The density of inquiline aphids (aphid/gall) was lower in larger galls, with no difference in slope among treatments (Table 1, line 38; Figure S4 in Appendix).

Hypothesis 1b - ***Aphids interfere with parasitism of the galler B. dracunculifoliae by attracting ants.***

There was a negative correlation between parasitism of the galler *B. dracunculifoliae* and number of terminal buds occupied by aphids but the slope did not differ among treatments (Table 1, line 78; Figure 2). Parasitism rate was positively affected by ant presence with differences between treatments (Table 1, line 72; Figure 3). The slope of the relationship was greater in control than in treatment hatched excluded ($z= -2.800, p=0.005$). Parasitism showed no significant relationship with ant richness (Table 1, line 69). Ant presence on branches was affected by aphid presence on branches with difference among treatment (Table 1, line 27). The slope was negative in Control and Hatched Gall Exclusion treatment and positive in Live Gall Exclusion (Table 1, line 27; Figure S5). Fewer galls were occupied by aphids in the Ant Exclusion treatment than in Control (Table 1, line 94). The Ant Exclusion treatment had no effect on aphid presence on branches (Table 1, line 11), or on parasitism rates of the galler *B. dracunculifoliae* (Table 1, line 41), and did not affect the relationship between parasitism and aphid inquilinism (Table 1, line 44).

Hypothesis 2 - ***Reducing the abundance of galler B. dracunculifoliae affects the species composition and abundance of aphids, ants, predators, and free-feeding herbivores, and host plant growth.***

Removing the commonest galler had no effect on predator richness, herbivore richness, herbivore abundance, aphid presence, ant richness, ant presence, or shoot growth (Table 1, lines, 1, 3, 6, 9, 12, 15 and 28 respectively). However, in the second week there was lower predator abundance in the Live Gall Exclusion treatment than in Control (Table 1, line 2; Figure S6 in Appendix).

Hypothesis 3a - ***Hatched gall interferes with parasitism rate of galler B. dracunculifoliae by lessening inquilinism.***

There was no significant relationship between parasitism of the galler *B. dracunculifoliae* and abundance of hatched galls (Table 1, line 75). However, parasitism on the galler *B. dracunculifoliae* was lower in the Hatched Gall Exclusion treatment than in the Control (Table 1, line 41). In addition, there was a positive correlation between abundance of hatched galls and inquilinism by the aphid, with no slope difference between Control and Ant Exclusion treatment (Table 1, line 93, Figure 4).

Hypothesis 3b - ***Hatched gall interferes with parasitism rate of galler B. dracunculifoliae by attracting aphids and ants that could interfere with parasitoids.***

The Hatched Gall Exclusion treatment had no effect on ant richness, ant presence, aphid presence, or inquilinism (Table 1, lines 12, 15, 11, and 94 respectively). However, there was a significant positive relationship between abundance of hatched galls and ant presence on branches in the Control (Table 1, line 95, Figure 5). There was also no significant correlation between abundance of hatched galls and aphid presence (Table 1, line 97).

Hypothesis 4 - ***Hatched galls, aphid colonies, and ants tending aphids interfere with abundance and parasitism of the apical galler R. limbata.***

There was a weak but significant negative correlation between G10 abundance and hatched gall abundance (Table 1, line 90, Figure S7 in Appendix). There was a positive correlation between *R. limbata* abundance and the number of branches with ants, and the slopes differ between treatments, being lower in the Hatched Gall Exclusion treatment (Table 1, line 96, Figure 6). Furthermore, there was no significant relationship between *R. limbata* abundance and aphid presence on branches (Table 1, line 82), and no difference in *R. limbata* abundance between the Control and Hatched Gall Exclusion treatment (Table 1, line 96). There was a positive correlation between parasitism of *R. limbata* and abundance of hatched galls, with no significant difference among treatments (Table 1, line 90, Figure 7). There was no significant correlation between parasitism of *R. limbata* and aphid or ant presence on branches (Table 1, lines 82, and 85).

Discussion

In chapter two, I revealed that NTI can modify interactions and offset or complement each other, creating pathways for feedback loops, and thus allowing a flexible modulation of species coexistence. Here I report the existence of even more NTI in the same system, and reveal some of the mechanisms through which they act to modulate trophic interactions. The fact that in a relatively discrete insect-plant community, consisting of seven groups of arthropods, interaction modification by NTI was so common suggests that it may also be widespread in nature.

This is an unprecedented effort to integrate the impacts of multiple NTI in structuring ecological communities. This is likely the first empirical study to perform concurrent manipulations at the community level under natural field conditions to study NTI mechanisms in detail. Most previous studies empirically demonstrating how NTI can be important for understanding community structure and dynamics have focused on much smaller subsets of natural communities, frequently under isolated conditions either in the field or in simplified laboratory mesocosms (Knight et al. 2005, van Veen et al. 2005, Bukovinszky et al. 2008, Ohgushi 2008, Hammill et al. 2015, Sanders et al. 2015, Wetzel et al. 2015). The only possible exception are the pioneering works of Kéfi et al. (2015, 2016) who used large datasets drawn from a combination of different experimental and observational studies and from the literature, to map positive and negative NTI onto trophic links (Kéfi et al. 2016). Despite studying a far larger community than studied here, their approach is far less detailed compared to the level explored here. For instance, the links are not all experimentally verified, and crucially they do not consider interference among non-trophic interactions (four-way interactions). The greatest challenge is to map the NTI onto each other, to account for interactions among them modifying each other. Whether or not it is possible to map a network including all the possible NTI, however, is yet unknown.

Not all the mechanistic pathways that I hypothesised here were corroborated by the results. I found that the inquiline aphid interferes with parasitism of the galler *B. dracunculifoliae* (thereby benefiting the galler) colonising preferentially parasitized galls and killing the parasitized larvae (hypothesis 1a), but not through attracting ants (hypothesis 1b). I could not find any direct effect of excluding the galler *B. dracunculifoliae* on the other groups, but there were effects on interactions between pairs of groups (hypothesis 2). Hatched galls increased parasitism rate of the galler *B. dracunculifoliae*, but it was not possible to determine the pathway since it was not through lessening inquilinism by aphids on live galls (hypothesis 3a), or through attracting aphids and ants that could interfere with parasitoids (hypothesis 3b). In fact, hatched galls increased inquilinism by aphids on live galls. Furthermore, hatched galls affected the galler *R. limbata* negatively (hypothesis 4), apparently through increasing parasitism, while ants affected the galler *R. limbata* positively but not when hatched galls were excluded. Aphid colonies had no significant effect on the abundance or parasitism of the galler *R. limbata*.

Most studies focus on single interactions but in multi-trophic systems indirect effects can link multiple interactions in a community (Strauss 1997, Jones et al. 1998, Knight et al. 2005, Ohgushi 2005, Bukovinszky et al. 2008, Prasad & Snyder 2010, Sanders et al. 2015, Hammill et al. 2015). These results demonstrate how different interactions combine to create net effects on population densities, and are further evidence for how NTI can cement species into a complex network (chapter 2). Below I discuss the hypotheses and results in detail before returning to the whole system at the end of the discussion.

Hypothesis 1 - ***Aphid interference with parasitism rate of the galler B. dracunculifoliae***

a) Inquiline aphids killing parasitised nymphs - Contrary to my prediction that it would reduce parasitism and to the results presented in chapter two, here I found a positive association between parasitism rates of the galler *B. dracunculifoliae* and inquilinism by aphids. There were more aphids in parasitised galls. Since parasitism happens first, in the early stages of gall development (Espírito-Santo & Fernandes 2002), parasitism must affect aphid choice of galls to colonise and not the other way around. I hypothesised that aphids would prefer larger galls and since parasitism was thought to increase gall volume (Espírito-Santo & Fernandes 2002), aphids would have a directed effect on parasitism. However, unlike in Espírito-Santo & Fernandes (2002), I found no evidence that parasitism increases gall volume. It may be that parasitised galls have their shapes altered and therefore are less well-sealed than non-parasitised ones (Espírito-Santo & Fernandes 2002, Espírito-Santo et al. 2004), facilitating aphid colonisation. In addition, there were significantly more aphids per gall in smaller galls than in larger ones. It is unclear whether the aphids affect gall development by consuming sap assimilates (see Fagundes et al. 2005, Neves et al. 2011), or, on the contrary, if aphid chooses smaller galls to colonise. Either way, parasitism measured from rearing galls would appear to be higher in larger galls simply due to lower aphid abundance and therefore lower parasitoid mortality. That would explain the pattern reported in Espírito-Santo & Fernandes (2002) of correlation between parasitism and gall volume.

The positive relationship between parasitism and inquilinism by aphids was due to aphids colonising preferentially parasitised galls. In reality, aphids affected parasitism negatively. The mortality of both parasitised and non-parasitised nymphs was higher in galls occupied by aphids, but since aphids colonised parasitised galls more frequently than non-parasitised ones, aphids affected parasitoids more intensely than the galler, helping control parasitism. In chapter two, the parasitism-inquilinism relationship was negative most likely because parasitism was measured from gall rearing and therefore did not quantify the dead parasitised nymphs that remained inside the galls. Parasitoid rearing represents the net success of parasitoids, but not the effective parasitism rate. Here, I measured parasitism through dissecting galls. The aphid preference for parasitised galls illustrates how an NTI (aphid-parasitoid) can regulate pairwise interactions (parasitoid – galler; van Veen et al. 2005, Hammill et al. 2015, Sanders et al. 2015). In addition, because parasitoids appear to facilitate aphid colonisation of galls, they benefit a species that affects them negatively, creating a feedback loop through NTI. The galler also competes with aphids for the shelter within the gall that they induce, but at the same time the galler is also benefited by aphid inquilines controlling parasitism. In sum, the NTI modification and feedback loops regulate species densities, allowing species that interact antagonistically to coexist (see van Veen et al. 2005, Sanders et al. 2015).

b) Aphids on branches attracting ants that interfere with parasitoids - Parasitism rate on the galler *B. dracunculifoliae* was lower on plants with more terminal buds occupied by aphids supporting my hypotheses that aphid presence on branches affects parasitism of *B. dracunculifoliae*. However, contrary to my prediction, ant presence on branches was negatively correlated with aphid presence in the Control and Hatched Gall Exclusion treatment, suggesting that aphids do not increase the presence of ants on the plants as assumed by Neves et al (2011). The ant-aphid relationship, nonetheless, was positive in the Live Gall Exclusion treatment. The reason for this may be that ants carry or lead aphids into galls where they are protected and have better resources, as ants have been recorded tending aphids inside galls (MB *pers. obs*). Even though there was no relationship between aphid inquilinsim and ant presence on branches, in the Ant Exclusion treatment there were fewer galls occupied by aphids than in control, suggesting that ants directly or indirectly promote inquilinism by aphids. On plants without *B. dracunculifoliae* galls aphids would have to remain on the branches, hence the positive relationship with ant presence on branches. In addition, contradicting my hypothesis and previous works (Seibert 1993, Fagundes et al. 2005, Neves et al. 2011), there was higher parasitism rate on plants with more branches occupied by ants. In the Hatched Gall Exclusion treatment this ant-parasitism relationship was negatively affected, however, indicating that hatched galls would be involved in promoting the positive effect of ants on parasitism. On the other hand, ant exclusion did not affect the presence of aphids on branches, parasitism rate of galler *B. dracunculifoliae*, or the correlation between parasitism of the galler *B. dracunculifoliae* and aphid inquilinism. It seems that aphids do not affect parasitism of the galler *B. dracunculifoliae* through attracting ants as previously thought (Fagundes et al. 2005, Neves et al. 2011). It is likely that an interaction cascade takes place in which aphids affect parasitism negatively, and ants negatively affect aphid presence on branches by leading them into galls. The more ants there are, the fewer aphids there will be on branches, and therefore the greater the parasitism. However, ants by increasing aphid inquilinism would also be affecting parasitism negatively. This is another example of how NTI can interfere with each other and moderate effects on species densities (see van Veen et al. 2005, Hammill et al. 2015, Sanders et al. 2015). This complementarity of NTI effects seems to be key to the formation of feedback loops that lead to species coexistence.

Hypothesis 2 - ***Reducing abundance of B. dracunculifoliae affects richness, abundance, and interactions of other species***

I expected that changes in abundances of other groups following gall removal would give an indication of a potential pathway through which the effect of the manipulation performed in chapter one could spread, affecting the whole community. However, the Live Gall Exclusion treatment affected interactions (link modulation) more than abundances of groups directly (node modulation). Live Gall exclusion had no direct effect on predator, herbivore, aphid, ant, or shoot growth. Only in the second week there was lower predator abundance in treatment live gall excluded than in control. It is possible that the manipulation may have had an immediate effect on predators, which then disappeared in the following weeks. The results in chapter two suggest that shifting interactions through NTI may reduce the impact on the nodes, and thus promote stability in the system (see van Veen et al. 2005, Hammill et al. 2015, Sanders et al. 2015). The Live Gall Exclusion treatment also changed the slope of the relationship between ant presence and aphid presence. Directly or indirectly live *B. dracunculifoliae* galls are key to the relationships among species in this system. Perhaps when galls are excluded the ant species composition changes to include more species that do not interfere with herbivores.

Hypothesis 3 - ***Hatched gall interference with parasitism rate of galler B. dracunculifoliae***

There was no correlation between parasitism of *B. dracunculifoliae* and abundance of hatched galls in this study. However, the treatment hatched gall exclusion presented lower parasitism rate than control, suggesting that hatched galls affect parasitism positively.

a) *Lessening the colonisation of live galls by aphids*

The results do not support the hypothesis that hatched galls increase survival of parasitized galling larvae by lessening the colonisation of live *B. dracunculifoliae* galls by aphids. Contrary to my prediction, I found a positive relationship between hatched galls and aphid inquilinism. In a previous work (chapter 2) though, the hatched gall – aphid relationship could be positive or negative at different moments depending on the abundance of hatched galls. In addition, as mentioned before, parasitism was positively related to aphid inquilinism, even though mortality of parasitised nymphs was significantly higher in galls occupied by aphids. At the same time that hatched galls seem to increase parasitism, apparently through some mechanism involving ants (see above), hatched galls also seem to increase inquilinism, which reduces parasitism. Thus, hatched galls seem to have opposite effects through different NTI.

b) Increasing aphids and ants on branches that interfere with parasitoids

Hatched galls showed a positive relationship with ant presence on branches, suggesting that hatched galls increase the presence of ants on branches for some as yet unknown reason. Here, parasitism of *B. dracunculifoliae* was positively related to ant presence on branches. However, ants would also increase inquilinism, since ants lead aphids into galls, which in turn increases parasitoid mortality and decreases parasitism. Therefore, it seems that the negative relationship between hatched galls and parasitism of the galler *B. dracunculifoliae* could be driven by NTI involving ants and aphids. Alternatively, it may be that hatched galls serve as a clue for parasitoids to identify suitable hosts (see Hassell 2000), but this could not be tested with the current experiments.

Hypothesis 4 - ***Hatched galls B. dracunculifoliae, aphid colonies, and ant interference with the apical galler R. limbata***

The results show that the abundance of the galler *R. limbata* was higher on plants with fewer hatched galls, and more ants, but was unrelated to aphid presence on branches. Ants could benefit the galler *R. limbata* by moving aphids into *B. dracunculifoliae* galls and also by interfering with parasitoids (Seibert 1993, Fagundes et al. 2005, Neves et al. 2011), although there was no effect of ants on parasitism of *R. limbata*. However, in the Hatched Gall Exclusion treatment the positive relationship between ants and the galler *R. limbata* disappeared, which indicates that the effect relies on hatched galls. The abundance of hatched galls was positively associated with parasitism of the galler *R. limbata*. This and the fact that exclusion of hatched galls changes the ant-galler *R. limbata* interaction abundance indicates that hatched galls directly or indirectly interact with the galler *R. limbata*. It seems that hatched galls interfere with the galler through increasing parasitism and also affecting the positive effect of ants on the galler *R. limbata*. Thus, the results here do not clarify how removing the galler *B. dracunculifoliae* in chapter one increased parasitism rates of the galler *R. limbata*. This may be due to the fact that here we quantified parasitism through dissection instead of gall rearing, and we also removed galls more frequently.

Conclusion

Very few studies have investigated the integrated effects of multiple interactions in structuring ecological communities (Knight et al. 2005, van Veen et al. 2005, Bukovinszky et al. 2008, Ohgushi 2008, Hammill et al. 2015, Sanders et al. 2015, Wetzel et al. 2015, Kéfi et al. 2015, 2016), none of which have assessed multiple NTI mechanism concomitantly at a community level or in such depth as presented here. One of the reasons for the lack of studies similar to this is that detecting and measuring indirect interactions in natural communities is an enormous challenge (Wootton 1994, Abrams 2008; Novak & Wootton 2008). Despite my best efforts to elucidate the pathways involved in the interactions among the various arthropod groups in the studied system, overall, the results reveal that, in fact, mechanistically explaining how precisely indirect interactions take place may be unfeasible even in relatively simple systems. NTI pathways in a network can quickly become very complex. All species and interactions are interlinked with the results that exploring any apparent simple interactions in isolation does not give the full story. In addition, interactions seem to be conditional on the presence and density of other species (context-dependent), which furthermore varies in time (Chamberlain et al. 2014, Poisot et al. 2014, chapter 2). Therefore, it is difficult to understand how one species affects another when the end effect is a combination of several influences from many species. Surely, studying ecological pairwise interactions in isolation can still be a valid approach depending on the question being addressed. In general, we have made important progress in understanding ecological processes using pairwise networks (e.g., Henneman & Memmot 2001, Lewis et al. 2002). However, I propose that rather than focusing on the outcome of specific pairwise interactions, for example, how aphids affect parasitism of the galler *B. dracunculifoliae*, a community or system based approach might be a way forward (Fontaine et al. 2011, Wootton 1994; Kéfi et al. 2012). The focus would then be on determining which internal mechanisms allow the system as a whole to adapt to a specific manipulation such as the removal of a galler species. Whether such an approach is feasible in the field remains to be tested. Advancing our understanding of such mechanisms could elucidate the underlying causes of stability and persistence of ecological communities and increase our ability to predict how they might respond to perturbations.

Table

Table 1. Summary of results of statistical tests. The values highlighted in bold are statistically significant ($P < 0.05$). G7 is the galler *B. dracunculifoliae,* and G10 is the galler *R. limbata*.

Line	**Dataset**	**Response**	**Explanatory variable**	χ^2	**d.f.**	**p**	**Post hoc comparison**
1	*Observation*	Predator richness	Treatment : Week	26.937	15	**0.029**	In week 6, Hatched < Ant exclusion (Tukey's HSD, p = 0.030)
2		Predator abundance	Treatment : Week	40.055	15	**<0.001**	In week 2, Control > Ant exclusion (Tukey's HSD, p = 0.034), and Live gall excluded (p = 0.008)
3		Herbivore richness	Treatment : Week	20.063	15	0.169	
4			Week	4.180	5	0.523	
5			Treatment	8.985	3	**0.029**	Ant exclusion > Hatched gall excluded (Tukey's HSD, p = 0.012)
6		Herbivore abundance	Treatment : Week	18.705	15	0.227	
7			Week	7.364	5	0.194	
8			Treatment	8.380	3	**0.038**	Ant exclusion > Hatched gall excluded (Tukey's HSD p = 0.028)
9		Aphid presence	Treatment : Week	3.291	3	0.348	
10			Week	152.800	1	**<0.001**	
11			Treatment	0.404	3	0.939	
12		Ant richness	Treatment : Week	4.700	2	0.095	
13			Week	45.383	1	**<0.001**	
14			Treatment	0.039	2	0.980	
15		Ant presence	Treatment : Week	5.481	2	0.064	
16			Week	62.948	1	**<0.001**	
17			Treatment	0.321	2	0.851	
18		Predator richness	Ant presence : Treatment	8.403	2	**0.014**	t = -2.54; Slopes comparison: Control < Live gall exclusion (p = 0.05)
19		Predator abundance	Ant presence : Treatment	14.658	2	**<0.001**	t = -1.963; Slopes comparison: Hatched gall excluded < Live gall exclusion (p < 0.001)
20		Herbivore richness	Ant presence : Treatment	1.724	2	0.422	
21			Ant presence	0.671	1	0.412	
22			Treatment	2.269	2	0.321	

23		Herbivore abundance	Ant presence : Treatment	1.706	2	0.426	
24			Treatment	0.882	2	0.643	
25			Ant presence	0.337	1	0.561	
26		Aphid presence	Ant presence : Treatment	21.192	2	**<0.001**	t = -1.480; Slopes comparison: Control < Live gall exclusion (p = 0.022)
27		Ant presence	Aphid presence : Treatment	26.030	2	**<0.001**	t = -0.670; Slopes comparison: Control < Live gall exclusion (p < 0.001)
28		Shoot growth	Treatment	0.952	1	0.329	
29	*Dissection*	Aphid per gall	G7 Parasitism : Treatment	3.899	2	0.142	
30			Treatment	3.920	2	0.140	
31			Parasitism	19.042	1	**<0.001**	t = 6.005
32		Aphid per gall	G7 Parasitism (binary) : Treatment	2.374	2	0.305	
33			Block	7.948	15	0.925	
34			Treatment	2.173	2	0.337	
35			G7 Parasitism (binary)	6.144	1	**0.013**	t = 2.630
36		Aphid per gall	Gall volume: Treatment	1.137	2	0.566	
37			Block	17.703	15	0.278	
38			Gall volume	6.602	1	**0.010**	t = -2.586
39			Treatment	7.264	2	**0.026**	
40		G7 Abundance	Treatment	2.351	2	0.308	
41		G7 Parasitism	Treatment	7.805	2	**0.020**	Control > Hatched gall exclusion (z = 2.834; df = 1; p = 0.004)
42			Block	32.614	15	**0.005**	
43		G7 Parasitism	Aphid per gall : Treatment	2.973	2	0.226	
44			Treatment	3.551	2	0.1669	
45			Aphid per gall	29.857	1	**<0.001**	
46		G7 volume	G7 Parasitism : Treatment	1.051	2	0.591	
47			Treatment	0.042	2	0.979	
48			G7 Parasitism	0.855	1	0.355	
49		G7 volume	G7 nymphs per gall : Treatment	10.317	2	**0.005**	t = 2.061 Slopes comparison: Control < Ant exclusion (p = 0.003)

50		G7 volume	Aphid per gall : Treatment	1.031	2	0.597	
51			Treatment	8.316	2	0.841	
52			Aphid per gall	0.345	1	**0.003**	z = -2.872
53		Parasitoid mortality	Inquilinism (binary) : Treatment	4.813	2	0.090	
54			Treatment	0.147	2	0.928	
55			Inquilinism (binary)	10.285	1	**0.001**	t = 3.465
56		G7 nymph mortaity	Aphid per gall : Treatment	3.380	1	0.184	
57			Treatment	0.360	2	0.835	
58			Aphid per gall	5.924	1	**0.014**	t = 2.520
59	*Observation + Dissection*	G7 Parasitism	Predator richness : Treatment	8.878	2	**0.011**	z = 2.819; Slopes comparison: Control > Ant excluded (z = -2.677; df = 1; p = 0.007), and Hatched gall excluded (z = -2.677; df = 1; p = 0.016)
60		G7 Parasitism	Predator abundance : Treatment	3.878	2	0.143	
61			Treatment	10.420	2	**0.005**	
62			Predator abundace	21.054	1	**<0.001**	z = 0.481; df = 1; p = <0.001
63		G7 Parasitism	Herbivore richness : Treatment	0.693	2	0.071	
64			Herbivore richness	1.837	1	0.175	
65			Treatment	6.964	2	**0.030**	
66		G7 Parasitism	Herbivore abundance : Treatment	5.834	2	0.054	
67			Herbivore richness	0.692	1	0.405	
68			Treatment	5.362	2	0.068	
69		G7 Parasitism	Ant richness: Treatment	0.088	1	0.766	
70			Ant richness	0.100	1	0.750	
71			Treatment	5.008	1	**0.025**	
72		G7 Parasitism	Ant presence : Treatment	8.137	1	**0.004**	z = 2.687; Slopes comparison: Control > Hatched excluded (z = -2.800; df = 1; p = 0.005)
73		G7 Parasitism	Hatched abundance : Treatment	0.018	1	0.893	
74			Treatment	0.986	1	0.320	
75			Hatched abundance	2.376	2	0.123	
76		G7 Parasitism	Aphid presence : Treatment	5.601	2	0.060	

77			Treatment	5.048	2	0.080	
78			Aphid presence	8.014	1	**0.004**	z = -2.811; df = 1; p = <0.004
79		G10 parasitism	Hatched abundance : Treatment	1.218	2	0.543	
80			Treatment	1.880	2	0.390	
81			Hatched abundance	4.768	1	**0.028**	z = 2.085; df = 1; p = 0.037
82		G10 parasitism	Aphid presence : Treatment	0.200	2	0.904	
83			Aphid presence	0.606	1	0.436	
84			Treatment	1.880	2	0.390	
85		G10 parasitism	Ant presence : Treatment	0.231	1	0.630	
86			Ant presence	0.182	1	0.668	
87			Treatment	1.415	1	0.234	
88		G10 abundance	Hatched abundance : Treatment	0.902	1	0.342	
89			Treatment	0.304	1	0.581	
90			Hatched abundance	4.134	1	**0.042**	z = -2.714, df = 1; p = 0.042
91		G10 abundance	Aphid per gall : Treatment	8.621	2	**0.013**	t = 1.327; Slopes comparison: Ant exclusion < Control (p = 0.034)
92	*Simple Linear Models*			**R**	**F**	**p**	
93		Aphid per gall	Hatched abundance	0.363	7.139	**0.003**	t = 3.079, df = 1, p = 0.004
94			Treatment	0.363	7.139	**0.003**	Ant exclusion < Control (t = - 2.387, df = 1; p = 0.024)
95		Ant presence	Hatched abundance	0.177	4.025	**0.044**	t = 2.006
96		G10 abundance	Ant presence : Treatment	0.228	2.66	**0.026**	t = 2.756, p = 0.010; Slopes comparison: Hatched exclusion < Control (t = - 2.150; df=1, p = 0.040)
97		Aphid presence	Hatched abundance : Treatment	NS		0.459	
98			Treatment	NS		0.873	
99			Hatched abundance	NS		0.722	

Figures

Figure 1. Relationship between parasitism rate and inquilinism by aphids in galls of *Baccharopelma dracunculifoliae* in distinct treatments, showing test of hypothesis 1a. See Table 1, line 43 for results of statistical tests.

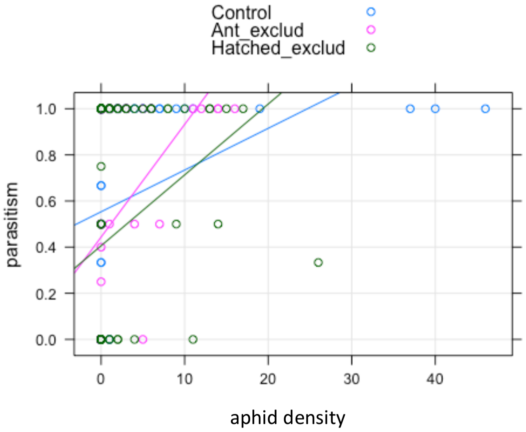


Figure 2. Relationship between parasitism rate of the galler *Baccharopelma dracunculifoliae* and frequency of branches occupied by aphids in distinct treatments, showing test of hypothesis 1b. See Table 1, line 78 for results of statistical tests.

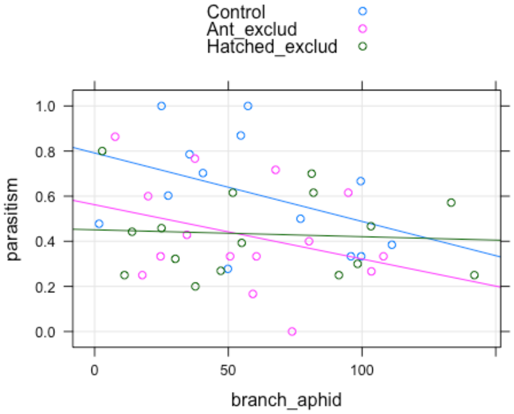


Figure 3. Relationship between parasitism rate on the galler of *Baccharopelma dracunculifoliae* and frequency of branches occupied by ants in distinct treatments, showing test of hypothesis 1b. See Table 1, line 72 for results of statistical tests.

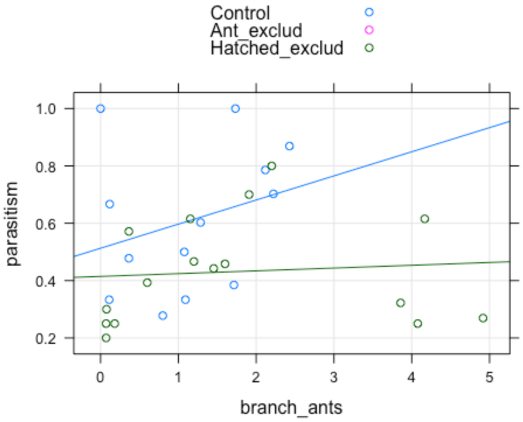


Figure 4. Relationship between the abundance of hatched galls of *Baccharopelma dracunculifoliae* and inquilinism by aphid in distinct treatments, showing test of hypothesis 3a. See Table 1, line 93 for results of statistical tests.

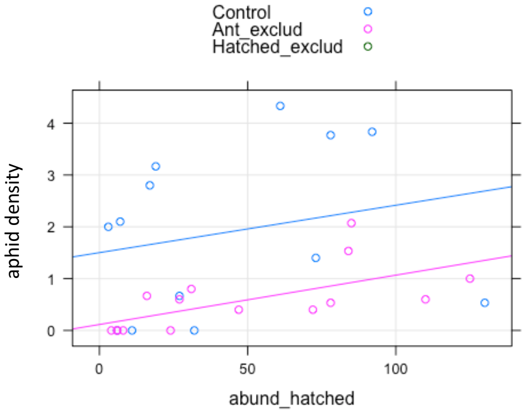


Figure 5. Relationship between abundance of hatched galls of *Baccharopelma dracunculifoliae* and ant presence on branches of plant individuals from Control, showing test of hypothesis 3b. See Table 1, line 95 for results of statistical tests.

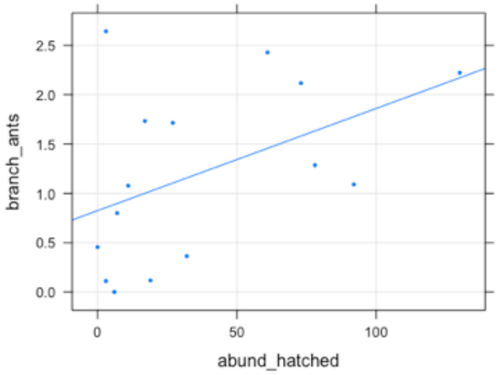


Figure 6. Relationship between abundance of the apical galler *R. limbata* and frequency of branches occupied by ants in distinct treatments, showing test of hypothesis 4. See Table 1, line 96 for results of statistical tests.

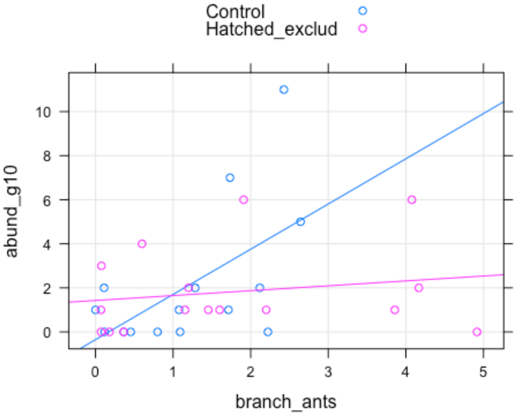
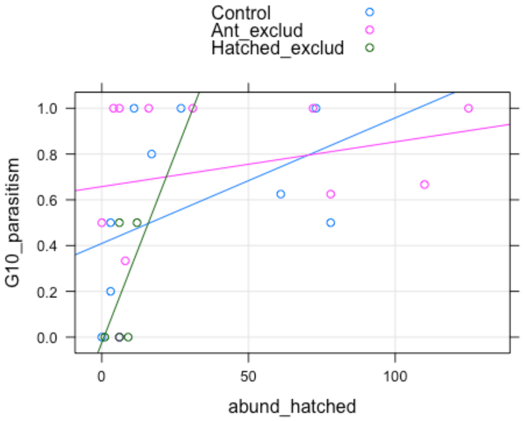


Figure 7. Relationship between abundance of the apical galler *R. limbata* and abundance of hatched galls of *Baccharopelma dracunculifoliae* in distinct treatments, showing test of hypothesis 4. See Table 1, line 90 for results of statistical tests.



Appendix

Figure S1. Number of inquiline aphids in parasitised and non-parasitised galls of *Baccharopelma dracunculifoliae,* showing test of hypothesis 1a. See Table 1, line 95 for results of statistical tests.

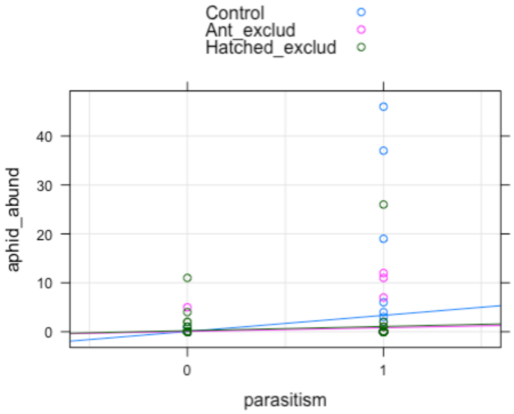


Figure S2. Mortality of parasitised nymphs of *Baccharopelma dracunculifoliae* between galls with and without inquiline aphids, showing test of hypothesis 1a. See Table 1, line 55 for results of statistical tests.

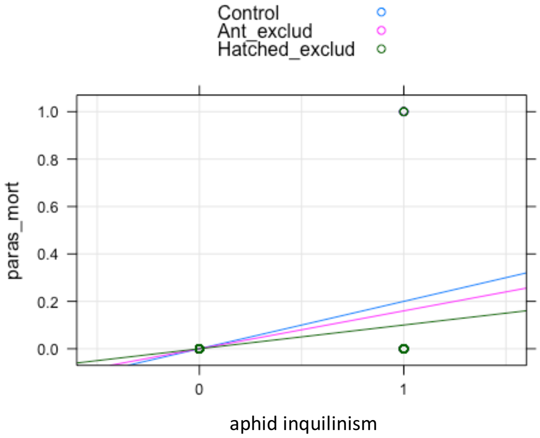


Figure S3. Mortality of non-parasitised nymphs of *Baccharopelma dracunculifoliae* between galls with and without inquiline aphids, showing test of hypothesis 1a. See Table 1, line 58 for results of statistical tests.

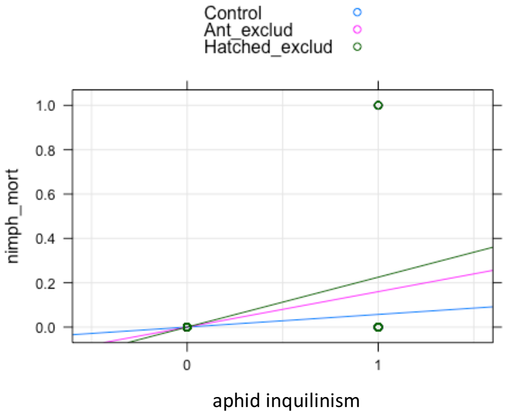


Figure S4. Relationship between the density of inquiline aphids and volume of galls of *B. dracunculifoliae*, showing test of hypothesis 1a. See Table 1, line 38 for results of statistical tests.

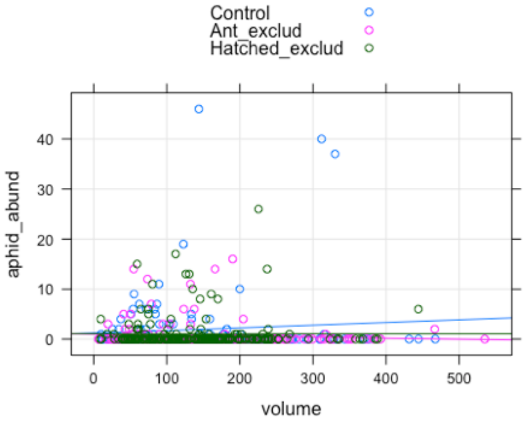


Figure S5. Relationship between frequency of branches occupied by ants and frequency of branches occupied by Aphids, showing test of hypothesis 1b. See Table 1, line 27 for results of statistical tests.

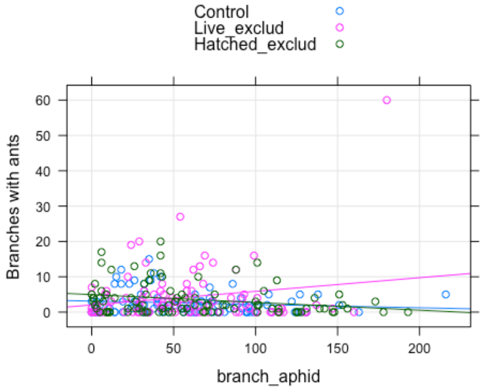


Figure S6. Predator abundance in different treatments: (a) across weeks and (b) on the second week only, showing test of hypothesis 2. See Table 1, line 2 for results of statistical tests.

a) b)

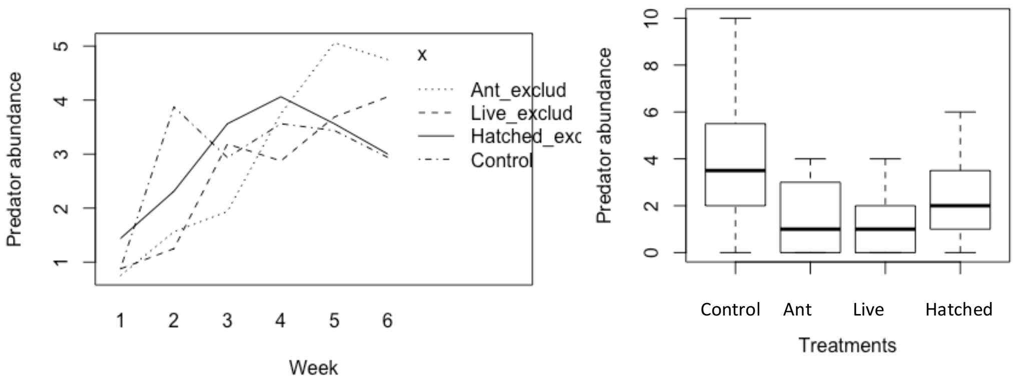
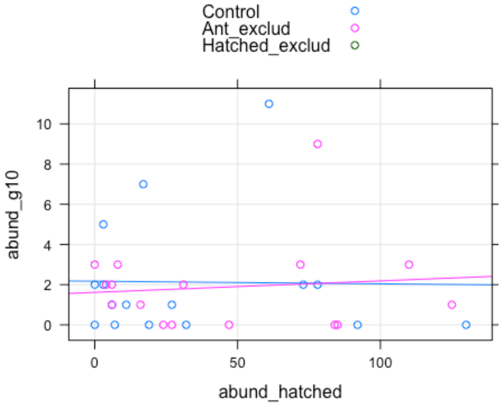


Figure S7. Relationship between abundance of the apical galler *R. limbata* and abundance of hatched galls of *B. dracunculifoliae*, showing test of hypothesis 4. See Table 1, line 90 for results of statistical tests.



CHAPTER 4

CHAPTER 4: Nature's deep web: Non-trophic interactions modifying interactions

Abstract

Ecologists have become increasingly aware of the importance of non-trophic interactions in ecosystem structuring and dynamics. However, there has been little empirical investigation of their significance at a community level under natural field conditions. Here, I performed experimental manipulations of species densities on a diverse and self-contained assemblage of arthropods associated with a major shrub species growing in a Brazilian savannah habitat. I removed live and hatched galls of the dominant species, as well as ants, from different plants. I quantified the changes in densities of several other species or guilds, as well as changes in direct interactions involving the galler, such as parasitism, and inquilinism by aphids. Specifically, I looked at how the density of certain pairs of groups related to each other, and how the relationship changed in relation to the presence or absence of a third group. This allowed the construction of an "effect network", with links categorised into two main types: node modulation, which are pairwise trophic and non-trophic interactions (effect on densities); and link modulation, which are three-way interactions (interaction modification), or four-way interactions (modification of an interaction modification). This effect network revealed the hidden web of indirect effects modifying the direct interactions and modifying each other. The removal of various different groups indirectly affect the relationships between several other pairs of groups to which the manipulated groups are not directly related. Ecological pairwise interaction networks cannot readily account for such phenomena. I propose a more holistic framework to study the role of indirect interactions in the structuring and dynamics of ecological systems.

Introduction

Understanding how communities become assembled and function over ecological time is one of the major concerns of community ecologists (Drake 1990, Rosindell et al. 2011). Investigating the role of biodiversity and structural complexity in the dynamics and stability of communities led many ecologists to search for patterns in food webs (description of trophic interaction among species), chiefly of predator-prey interactions (Solé & Montoya 2001, Dunne et al. 2002, Montoya et al. 2006, Rooney et al. 2006). However, food webs are only a subset of communities, which include many other kinds of direct and indirect, trophic and non-tropic interactions across multiple trophic levels (Ings et al. 2009, Olff et al. 2009). Although ecologists have become increasingly aware of the importance of non-trophic interactions (NTI) in commmunity structuring and dynamics (Bolker et al. 2003; Bruno et al. 2003, Sanders et al. 2014, Kéfi et al. 2012, 2015), there has been little empirical investigation of their significance at the level of whole ecological communities (Ohgushi 2008). The main difficulty in performing such studies is that networks can quickly become very complex, as in nature the number of indirect interactions can increase exponentially with the number of species involved (Patten, 1983). Therefore, there is a need to develop new theories and methods to integrate the different types of interactions at the scale of communities (Ohgushi 2008, Kéfi et al. 2012, 2015).

High-order interactions, that is, interactions involving modulation by other species (Wootton 1994, 2002), play an important role in stabilising communities (Kelsic et al. 2015, Hammill et al 2015). They can, for instance, attenuate negative interactions such as when nonprey species increase the persistence of predation-susceptible species by modifying the ability of predators to detect prey (Hammill et al. 2015); or consumer species reduce competition among their respective resource species and have a positive indirect effect on each other (Sanders et al. 2015). This way, high-order interactions can be key to species coexistence, promoting diversity (Wilson, 1992) and long-term persistence of ecological communities (Hammill et al 2015). In chapter two, I argue that communities may have a mechanism to keep populations relatively stable despite disturbances, at the expense of changes in the magnitude of interactions. If there is such an internal mechanism of community stability, NTI are likely to play an important role in it. Firstly, they seem to be widespread and can influence several groups cementing the assemblages. Secondly, they are context-dependent and regulated by feedbacks, which means that they will change their influence on interactions according to population densities (chapters 1 and 2). Thus, further investigation of non-trophic regulation could help unveil the mechanistic basis of community structuring and stability.

In chapters one and two, I performed experimental manipulation of species densities on the tropical shrub *Baccharis dracunculifolia* D.C. (Asteraceae), a highly diverse and self-contained model system, which hosts a diverse fauna of arthropods. The effects of manipulating single groups (chapters 1 and 2) on species densities were not very dramatic, they were more visible in their effect on interaction modification. Experimentally reducing the abundance of the commonest gall-making species associated with the host plant caused changes in structure and lowered robustness of the manipulated galler-parasitoid food webs, most likely through trophic links not included in the webs (e.g., shared predators) or non-trophically (e.g., through changes in habitat availability). In addition, hatched galls of the same dominant galler, which serve as habitat for other species, can trigger NTI that will feedback to the galler modifying its interactions with other species (chapters 1 and 2).

I hypothesised that due to the ubiquity of NTI, a complex network of NTIs could be constantly modifying interactions and shaping ecological communities at all times (chapter 2, but see Ogushi 2008). There would be an interdependence among species, and interaction outcomes would be a product of several influences. Consequently, I predicted that the manipulation of any group – or even of any of their by-products - could potentially affect any other group and modify unrelated interactions. Thus, here I investigated whether random changes in composition could affect the community as a whole. I performed further manipulations on the same system, excluding live and hatched galls of the dominant species, as well as ants, from different replicated plants. I quantified the changes in densities of several other species or guilds (hereafter groups) as well as changes in direct interactions involving the galler, such as parasitism, and inquilinism by aphids. Specifically, I combined direct observation and gall dissection data to look at how the density of certain pairs of groups relate to each other, and how the relationship changes in relation to the presence or absence of a third group. This allowed the construction of an "effect network", with links categorised into two main types: node modulation, which are pairwise trophic and non-trophic interactions (effect on densities); and link modulation, which are three-way interactions (interaction modification), or four-way interactions (modification of an interaction modification).

Methods

Study Site

This study was conducted at Serra do Cipó, in Minas Gerais state, southeast Brazil. This region is in the southern portion of the Espinhaço Mountain Chain, in the Cerrado biome and is characterised by quartzitic soils covered by rocky grasslands, with a predominance of herbs and shrubs (Fernandes 2016). It has a Cwb Köppen climate type, with dry winters and rainy summers (Schulz & Machado 2000). The average annual rainfall is between 1250 and 1550 mm, and the average temperature ranges from 18 to 19ºC (Madeira & Fernandes 1999). The study site (19°16'48"S - 43°35'20"W; 1170 m elevation) is undergoing restoration with native species since 2010, after serving as a source of soil for the paving of the MG-010 highway. The plant species *Baccharis dracunculifolia* D.C. (Asteraceae) is one of the species planted and is now dominant in the area (see Fernandes et al. 2015).

The study system

The plant species *B. dracunculifolia* is a perennial, evergreen, dioecious shrub, 2-3 m in height, which is widely distributed across southern and central South America (Espírito-Santo et al. 2003). *B. dracunculifolia* frequently forms well-defined patches 18 to 12,000 m2 in area (Collevatti & Sperber 1997). It has a key role in natural succession and regeneration (Fernandes et al. 2016) and is, therefore, important in terms of biodiversity and ecosystem functioning. The plant species hosts a diverse fauna of free-feeding herbivores, mostly Hemiptera, Coleoptera and Orthoptera, and also many predators including the orders Araneae, Coleoptera, Matodea, and Hymenoptera. Among the 17 species of gall-inducing insects recorded on *B. dracunculifolia* in multiple locations (Fernandes et al. 1996, 2014), *Baccharopelma dracunculifoliae* (Sternorrhyncha: Psyllidae; galler G7 in chapter 1) is the commonest (Araújo et al. 1995). It induces a gall in the midrib of the leaf, which bends over itself until the borders are joined, forming an elliptical, green, glabrous, single-chambered gall that usually harbours up to four nymphs (Lara & Fernandes 1994). The galls remain attached to the plant after dehiscence and gradually become dry and woody (Espírito-Santo & Fernandes 1998). Both hatched and non-hatched galls of *B. dracunculifoliae* are occupied by many inquiline invertebrates, such as ants, spiders, aphids, etc. (Collevatti & Sperber 1997, Espírito Santo & Fernandes 2002). At least ten parasitoid species have been reared from gall of *B. dracunculifoliae* (chapter 1) and parasitism rates are around 45% (Espírito-Santo & Fernandes 2002). When the parasitoids emerge, they leave a characteristic exit hole on the gall wall (Espírito-Santo et al. 2004), facilitating aphid colonisation of live galls (MB *pers. obs.*).

The aphid *Uroleucon tucumani* (Sternorryncha: Aphididae) is by far the most frequent inquiline (a species that occupy a living space produced by another species, such as a gall) and can indirectly kill the nymphs of the gall maker (Collevatti & Sperber 1997, Fagundes et al. 2005, MB *pers. obs.*). This aphid species also feeds and reproduces on the apical meristems of the host plant, forming dense colonies that produce honeydew (sugary secretions). *U. tucumani* attracts at least 15 species of ants, which tend and protect them in a trophobiotic relationship (Fagundes et al. 2005, Neves et al. 2011, MB *pers. obs*). It has been found that the presence of ants reduces the number of *B. dracunculifoliae* nymphs per gall, and the presence of aphids reduces *B. dracunculifoliae* gall size because aphids compete with the galler for sap assimilates and young leaves in terminal buds (Fagundes et al. 2005). Neves et al. (2011) observed that the presence of ants and aphids on *B. dracunculifolia* decreased the abundance of other free-feeding herbivores, and that the presence of aphids decreased plant shoot growth. Ants tending aphids can have a direct negative impact on herbivores (Abe 1988, Fernandes et al. 1999). However, the aphids on their own can also reduce the abundance of fluid-sucking and chewing insects due to exploitation competition or by altering the nutritional quality of the host plant (Fay et al. 1996, Larson & Whitham 1997).

Experimental design

Sixty-four isolated individuals of the plant species *Baccharis dracunculifolia* D.C. (Asteraceae) of 1.5-2.0m in height, distant at least 5m from conspecific plant individuals but in the same area were randomly identified and marked in the field. The plants were randomly assigned to four treatment groups (16 plants each) in blocks at least 20m apart from each other. Thus, there was one plant individual for each treatment in each block, and 16 blocks altogether. Different manipulations were performed in each treatment according to the hypothesis being addressed:

(i) *Ant Exclusion* - Ants were excluded by applying a non-toxic resin (Tanglefoot®, Tanglefoot Company, Michigan, USA) to the basal stem of the plants. During the study period, the plant individuals were monitored twice a week to check the effectiveness of the treatment, which was repeated where necessary;

(ii) *Live Gall Exclusion – B. dracunculifoliae,* the commonest galling species found on *B. dracunculifolia* was removed by direct collection. Since new galls could be induced over the monitoring period, the treatments were maintained by excluding newly induced galls every week, which prevented any galls from reaching full development;

(iii) *Hatched Gall Exclusion* - All hatched galls of *B. dracunculifoliae* were excluded from each of 16 plant individuals. Weekly observations were performed to exclude newly hatched galls.

(iv) *Control* – Non-galled leaves were removed in an equal amount to the average number of galls collected in treatments (iii) Live Gall Exclusion and (iv) Hatched Gall Exclusion below;

Monitoring and data collection

During the study period (August - September 2015), I combined direct observation and gall dissection data to quantify densities of several arthropod groups in each treatment as well as the frequency of direct interactions, including parasitism and inquilinism.

Observations – I performed weekly observations on the plant individuals during the study period. The species and abundance of arthropods on isolated plants were quantified by directly counting individuals and morphospecies for 10 minutes per week (between 9:00am and 3:00pm), totalling one hour per plant over the two months. I quantified the number of individuals of each species (or morphospecies) of ants, predators and free-feeding insect herbivores, as well as the number of branches occupied by ants (ant presence), and terminal buds occupied by aphids (aphid presence). During the study period, at least one individual of each morphospecies was collected for identification. Plant shoot growth was also measured using a tape measure for treatments Control and Live Gall Exclusion to test the effect of the galler on plant growth. On the final week of the eight-week study period, I quantified the abundances of *B. dracunculifoliae* galls, and hatched *B. dracunculifoliae* gall (except in the treatments in which they had been excluded). I counted all full-sized or close to full-sized live galls and all hatched galls of *B. dracunculifoliae* found in three half-meter branches haphazardly chosen around the crown of each individual of *B. dracunculifolia*. I also quantified the abundance of an apical gall induced by *Rachiptera limbata* Bigot (Diptera: Tephritidae – galler G10 in chapter 1) on the same branches. I chose to include the galler *R. limbata* in the study because in a previous experiment (chapter 2), there was a twofold increase in the frequency of one of the parasitoid species (Bracon sp2) attacking *R. limbata* after removing the galler *B. dracunculifoliae*.

Gall dissection – After counting the aforementioned galls, I collected 15 of each gall type, *B. dracunculifoliae* and *R. limbata* (five of each type per branch). They were stored individually and taken to the lab for dissection. To calculate parasitism rates I dissected the galls and quantified the proportion of parasitised and unparasitised nymphs per gall through the presence of "mummies" (parasitised galler nymphs). I also determined parasitism rates and aphid inquilinism (aphids per gall; not to be confused with "aphid presence" on branches) of the galler *B. dracunculifoliae*. Before dissection, galls were also measured for width and length to calculate their volumes - as an indicator of performance - according to their shapes (Volume = $4/3\pi$ [1/2 Length] $[1/2\text{Width}]^2$, for ellipsoid galls), as in Dunham (1983) although parasitism is thought to increase gall size (Espírito-Santo & Fernandes 2002).

Statistical analyses

Observation - I used data from the weekly observations to compare treatments in terms of the species and abundance of ants, predators and free-feeding insect herbivores, as well as the number of branches occupied by ants (ant presence), terminal buds occupied by aphids (aphid presence), and shoot growth. In each case, I fitted generalised linear mixed effect models (GLMMs; Crawley 2013) or, whenever it was not possible to obtain a satisfactory model fit, I fitted linear mixed effect models (LMMs) after square-root or log-transforming the data. I used the lme4 package (Bates et al. 2014) in R (R Core Team 2014) to fit the models. I entered treatment as a fixed effect, week as a covariate and plant individual as a random effect in all GLMM models. I used the testInteractions function (phia Package) to perform Wald chi-square test for *post hoc* comparisons between treatments. To test the effect of ants on other groups, I also contrasted each of those response variables with ant abundance and ant presence entering treatment as a covariate with interaction, and week and plant individual as a random effect in each model. For the analyses of effects on interactions (link modulation) I looked for variation among treatments in the slope of the relationship between pairs of groups – e.g., how herbivore abundance varies with ant abundance in each treatment. I used the testInteractions function to perform the pairwise comparisons of adjusted slopes with respect to the response variable for contrasts of the factor treatment (De Rosario-Martinez 2013).

I checked the GLMMs models for over-dispersion of residuals using the function overdisp.glmer (RVAideMemoire Package). To correct for over-dispersion I refitted the models with negative binomial distribution using the function glmer.nb (MASS Package) instead of glmer. To determine the structure of the random effect in the models I compared models allowing for variation in intercept within random effect to those allowing for variation in intercept and slope and selected the ones with lower Akaike Information Criteria (AIC) score. I performed simplifications of the maximal models by removing non-significant fixed effects to obtain a minimum adequate model (Crawley 2013). P-values of fixed effects were generated by likelihood-ratio tests of the full model with and without the explanatory variables. I then refitted the minimum adequate model using Restricted Maximum Likelihood (REML) and visually checked the residual plots for deviations from homoscedasticity or normality. No correction for multiple tests was done because the response variables were independent from each other.

Dissection - Using the dissection dataset I tested the relationships between inquilinism (aphid/gall) and parasitism; parasitism and volume of *B. dracunculifoliae* gall; volume of *B. dracunculifoliae* gall and nymphs per gall, and inquilinism; entering treatment as a covariate in all models. For all analyses of dissection data, I used block as a random effect. Minimum adequate models were obtained, and models were checked, as described above.

Observation plus dissection - In order to combine the observation and dissection datasets, the observation data had to be combined across weeks, therefore it was not possible to use mixed effect models. I fitted general linear models (GLMs) with binomial distribution for each response variable (parasitism of gallers *B. dracunculifoliae* and *R. limbata*) against the explanatory variables (species and abundance of ants, predators and free-feeding insect herbivores; ant presence; aphid presence; and abundance of hatched *B. dracunculifoliae* galls) in an interaction with treatment. I also used GLMs to compare treatments regarding gall abundance of *B. dracunculifoliae* and *R. limbata*, and plant shoot growth. I used ordinary linear models to test the relationship between abundance of *R. limbata* galls and abundance of hatched *B. dracunculifoliae* galls; inquilinism; ant richness, abundance and presence; as well as the relationship between inquilinism and abundance of hatched *B. dracunculifoliae* galls. All models had treatment as a covariate with interaction. Minimum adequate models were obtained, and models were checked, as described above. When necessary, the depended variables were square-root or log-transformed to improve the homoscedasticity of residuals.

Results

Throughout the entire two-month observation period I quantified 1,427 ants from 15 different species (Table S1) found on 988 branches, 1,109 predators from more than 9 morphospecies (spiders, for instance, were grouped together as a single morphospecies), 629 free-feeding herbivore insects from more than 41 morphospecies, and 22,564 terminal buds occupied by aphids. I quantified 1,346 hatched galls, and 1,260 live galls of the galler *B. dracunculifoliae*, of which 522 live galls were collected and dissected. I also collected and dissected 92 live gall of the galler *R. limbata*. The effects identified are portrayed in an effect network (Table1; Figure 1), and for the sake of simplicity the interactions and corresponding results of the statistical tests for each link in the network are shown in Table 2. From a total of 29 links in my network (Table 1, Figure 1) there were five trophic and five non-trophic pairwise links (node modulation); 12 three-way links (interaction modification), and five four-way links (modification of an interaction modification).

Ant effects

The presence of ants on branches negatively affected the richness and abundance of predators. However, excluding ants also reduced the richness and abundance of predators (link 5 - all link codes described hereafter refer to Table 1 and Figure 1). Ants were negatively related to the presence of aphids on branches (link 6). In the Ant Exclusion treatment the relationship between the number of galling nymphs and volume of *B. dracunculifoliae* galls was more positive (link 7), and the positive relationship between predator richness and parasitism of the galler *B. dracunculifoliae* (link 30) was weaker (link 8, four-way interaction). Ant presence was positively correlated with parasitism of the galler *B. dracunculifoliae* (link 9), as well as the abundance of the galler *R. limbata* on the plants (link 10). Ant exclusion lowered the number of inquiline aphids inside *B. dracunculifoliae* galls (link 11); and also weakened (link 12, four-way interaction) the positive relationship between aphid inquilinism and abundance of the galler *R. limbata* (link 13).

Aphid effects

Galls of *B. dracunculifoliae* with higher aphid inquilinism presented lower volume, and higher nymph mortality (link 14). Mortality of parasitised nymphs was also higher in galls with aphid inquilines (link 15). On plants with more branches occupied by aphid, parasitism of the galler *B. dracunculifoliae* was lower (link 16), and ants were less frequently observed (link 6).

Effect of hatched galls

Ants were more frequent on plants with higher abundance of hatched galls (link 17). Excluding hatched galls negatively affected (link 18) the positive relationship between predator richness and parasitism of the galler *B. dracunculifoliae* (link 29), reduced parasitism of the galler *B. dracunculifoliae* (link 19), and also weakened (link 20) the positive relationship between ant presence and parasitism of the galler *B. dracunculifoliae* (link 9). Plants with higher abundance of hatched galls also presented higher inquilinism (link 21), higher parasitism of the galler *R. limbata* (link 22), and higher abundance of the galler *R. limbata* (link 23). In the Hatched Gall Exclusion treatment, the correlation between ant presence and the abundance of the galler *R. limbata* (link 10) was weakened (link 24).

Effect of the galler B. dracunculifoliae

Excluding the galler *B. dracunculifoliae* reduced the abundance of predators, but only in the second week of the study period (link 25); it also positively affected (link 26) the relationship between ant presence and aphid presence on branches (link 6).

Parasitoid effects

In addition, aphid inquilinism (link 14) was positively affected by parasitism (link 27). Parasitism of the galler *B. dracunculifoliae* (link 2) was higher in plants with higher predator species richness and abundance (link 28).

Only herbivore richness and abundance on the plants (link 29) did not affect or were affected by any other group or interaction analysed.

Discussion

Whilst we already knew that indirect effects must be important for understanding community structure and dynamics (Wooton 1993, Ohgushi 2005, Knight et al 2005, Bukovinszky et al. 2008, Sanders et al. 2014, Kéfi et al. 2012, 2015, Hammill et al. 2015) this is thought to be the first truly empirical study to demonstrate, for an entire community, exactly how numerous non-trophic indirect effects can take place at the same time. Previous studies have traced specific pathways of indirect interactions connecting multiple species across trophic levels (Ohgushi 2005, Bukovinszky et al. 2008, Sanders et al. 2014, Hammill et al. 2015) and environments (Knight et al 2005, Prasad & Snyder 2010). For instance, Knight et al. (2005) provide empirical evidence that fish predation on dragonfly larvae in ponds indirectly facilitate terrestrial plant reproduction by reducing the negative effect of adult dragonflies on the abundance and behaviour of insect pollinators. Likewise, Bukovinszky et al. (2008) show that plant quality can affect a herbivore–parasitoid–secondary parasitoid food web via the propagation of trait and density-mediated effects. In a pioneering effort, Kéfi et al. (2015) have drawn up a more comprehensive rocky intertidal network including non-trophic interaction using large data sets from many different sources including the literature and observations, as well as experiments.

Here, however, I not only explored global effects taking place at a community level but experimentally tested each one of the interaction modifications identified. By performing several manipulations concomitantly under natural field conditions I was able to detect how frequently NTI interfere or modify other interactions. The manipulations revealed a hidden web of indirect effects modifying the direct interactions, as well as modifying each other. The removal of different groups indirectly affected the relationships between several other pairs of groups with which the manipulated groups did not directly interact. That indicated that somehow the species are indirectly connected. In a web including ten pairwise interactions, of which only five are trophic, this study revealed additional 19 potential NTI modulating nodes and modifying interactions. I identified several three-way and four-way interactions demonstrating how different effects combine to create net effects on species densities (see chapter 2).

There were several indirect NTI interfering with the pairwise trophic interaction between the galler *B. dracunculifoliae* and its parasitoids (Figure 1, link 2). For example, inquiline aphids can kill the galler *B. dracunculifoliae* nymphs (see Espírito-Santo & Fernandes 2002), but because they preferentially attacked parasitised galls, they can negatively affect parasitism and therefore also benefit the galler *B. dracunculifoliae*. As another example, ants, by leading aphids into galls, may positively affect parasitism on the galler *B. dracunculifoliae* by reducing aphids on branches. On the other hand, by increasing aphid inquilinism, ants also negatively affect parasitoids. Furthermore, hatched galls increase parasitism on the galler *B. dracunculifoliae*, perhaps serving as a clue for parasitoids (see Hassell 2000), but they also increase aphid inquilinism and therefore reduce parasitism on the galler *B. dracunculifoliae*. Finally, parasitism of the galler *B. dracunculifoliae* was also positively associated with predator richness and abundance, but the relationship was dependent on the presence of ants and hatched galls (four-way interactions). These results show that NTI can really overlap and interfere with each other creating total net effects that are non-linear and difficult to predict since they also change with time as population densities fluctuate (Menge et al. 1994, Chamberlain et al. 2014).

Similarly, there were several effects modifying the interaction between the aphid inquilines and the galler *B. dracunculifoliae*. Parasitism appeared to facilitate the interaction, possibly by changing gall shape and allowing aphids to get in through the longitudinal aperture (see chapter 3, Espírito-Santo & Fernandes 2002, Espírito-Santo et al. 2004). Ants also facilitated the interaction, perhaps by leading or even carrying aphids inside galls (MB *pers. Obs*). Hatched galls were also positively associated with inquilinism, but the effect was disturbed by ant presence on branches (four-way interaction). There are many other similar examples of three and four-way interactions in the network (described in detail in chapter 3). Apart from the interaction between free-feeding herbivores and the host plant, every direct interaction on the web seemed to be indirectly influenced by at least one of the other groups. In several cases, it was not possible to determine the mechanism behind the indirect links, even though the natural history of the system is fairly well-known (Espírito-Santo & Fernandes 2002, Espírito-Santo et al. 2004, Fagundes et al. 2005, Neves et al. 2011). I acknowledge that some links in the network represent mere correlations between densities of two groups, which does not mean that the groups actually affect one another. For instance, plants with more predators showed higher parasitism of the galler *B. dracunculifoliae*, which does not necessarily mean that the groups positively affect each other, since they may be both affected by a third group, such as the ants (Neves et al. 2011). However, I include these links firstly because it would be difficult to clarify whether that is the case, and second because the fact that the correlation changes in the absence of other groups, shows that the correlated groups are connected through a third group, therefore there is potential for indirect interaction, albeit via additional intermediate groups. The majority of links must be genuine NTIs, otherwise the manipulations would not have had such remarkable effects.

This level of detail on indirect non-trophic interactions for such a species rich multi-trophic system is truly unsurpassed.

Demonstrating such complex non-trophic interactions is intriguing, but can it really tell us how the web of interactions is organised? Investigating how precisely an effect takes place requires investigating the NTI modulating that effect (see Fontaine et al. 2011, Kéfi et al. 2012, Bairey et al. 2016), and even then it may prove difficult. For instance, here, despite the manipulations I was not able to clarify exactly how hatched galls affect parasitism rates. Unveiling the mechanism behind each single link on the web would require formulating and experimentally testing many more hypotheses (see chapter 3). It seems almost impossible to determine how a species affects another when we consider that all those non-trophic indirect effects may be taking place at the same time and interfering with each other (see Pintor and Soluk 2006, Sanders and van Veen 2011). In addition, the links represented in the web may vary in magnitude through time, or may even be transient (see chapter 2). As recent studies show, interspecific interactions are frequently context dependent (Chamberlain et al. 2014). The context is not only defined by the physical conditions but, more importantly, by the densities of each group involved and the indirect interactions modifying and overlapping with each other. In this sense, NTI can hugely increase the context-dependency of interactions. As reported in chapters two and three as well as in previous works (Veen et al. 2005, Sanders et al. 2015), NTI, by modifying interactions and offsetting or complementing each other, can allow a flexible modulation of species coexistence. It is probably clearer now that the indirect effects described in chapter one of removing the dominant galler from a galler-parasitoid food web in the same system could have indirectly spread through many different and unpredictable ways to affect web structure and stability. In chapter three I attempted to determine a mechanistic pathway for the effect in question but despite additional experimental tests it was not possible to precisely determine the mechanism.

At least in my web, it appears that considering pairwise interactions in isolation would be simply abstractions. The interaction does not take place outside the context of the web of indirect effects modifying it – unless isolated in an experimental setting or in computational models (Bonsall & Hassell 2000, Sanders et al. 2012, 2015). In the community I studied here, population densities at a given time seems to be influenced by the sum of several indirect effects taking place at that time. If this is the case for natural systems in general, no inference can be made on the magnitude or direction of a given interaction in nature by studying it in isolation. Communities seem to be a lot more intertwined than has usually been assumed (see Kéfi et al. 2015). Thus, it seems that describing species effects or roles in a system by depicting links in a web may be a misrepresentation. How else would it be possible to capture nature's complexity though? It would seem to require the development of new methodologies and hypotheses to shift the focus from pairwise interactions to more comprehensive approaches – e.g., mechanics of community assembly (Drake 1990, Levin 1998).

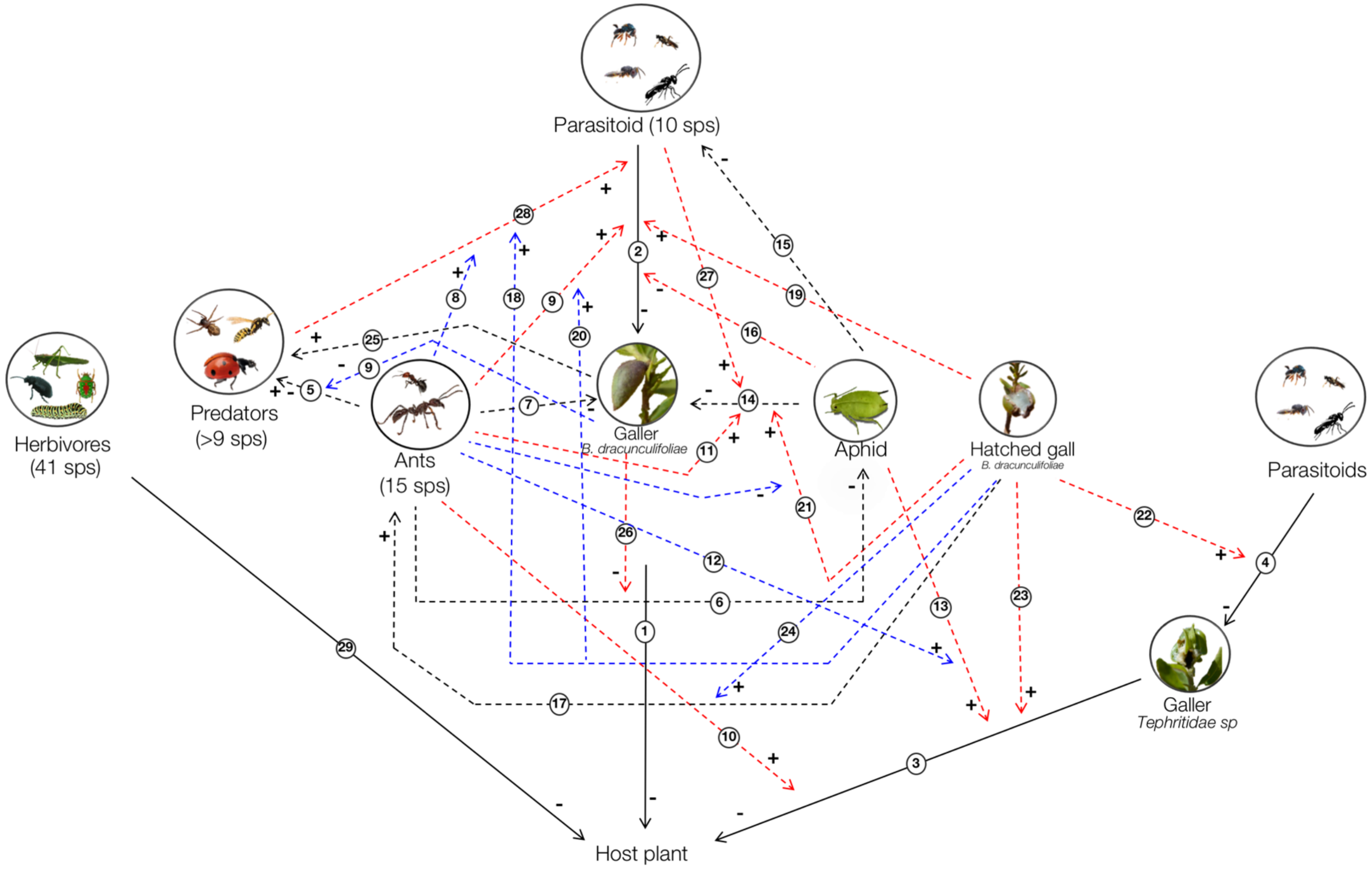
Ecologists have long recognised that ecological communities are complex adaptive systems (CAS; May 1973, Levin 1998). CAS can be defined as being composed of many independent components interacting locally and producing an organised and well-defined overall behaviour, independent of the internal structure of the components (Brown 1994ab, West 2006ab). There is a circular relationship between the structure of the global system and the local interactions between the components. The overall structure can be defined as the local network of all relationships, which is produced and maintained at a given moment by the total interactions that occur at that time. Each and every system component interact with their immediate neighbours, thereby modifying the overall structure. Since each component responds to the overall structure, the behaviour of each part is determined by the whole. At the same time, the independent response of all components in a given time produces the whole of the next moment. New properties emerge in a CAS, not because of changes in the basic nature of the components but as a result of the interactions among components (Levin 1998). For instance, the interaction between species A and B that should be linear if taken separately will be non-linear in nature because, through indirect effects, species A may, for instance, benefit a competitor, host, or prey, or affect an environmental condition that will in turn affect species B.

The manipulations of random groups that I have performed in the system so far seem to have produced changes that could not be predicted from analyses of pairwise interactions, since the effect will depend on the global context (species composition and abundances mainly) at each moment. This is a classic emergent property, typical of CAS. Thus, even though important progress has been made in community ecology through more reductionist approaches, we may still be unable to understand the whole (e.g., assembly rules) in the same way we understand the parts (e.g., pairwise interactions) due to emergent properties (Drake 1990, Levin 1998). In order to approach ecosystems as complex systems, in which the components interact and affect the whole and the whole affects the components back, one important step seems to be to summarise all different possibilities of interaction in terms of impacts on the global state of the system, which in turn affects all species. The indirect influence of one species on others has received many different descriptions according to the means by which they are mediated: density-mediated (Abrams et al. 2004), trait-mediated (Pearson 2010), habitat-mediated, facilitation (Bruno et al. 2003), competition, etc. In broad terms, these effects are all the same - indirect influence of one species on a certain aspect of the system that will affect other species - and can be grouped into a more general concept. Species can only affect each other through altering resources and conditions, which comprise the modern niche concept (Hutchinson 1957, Tilman 1982, Chase and Leibold 2003). Consequently, instead of focusing on single effects, perhaps we should focus on the idea that species interact through their global impacts on the multi-dimensional niche space, with an "output" vector whereby each species affects the whole structure, and an "input" vector through which the whole structure affects each species. I envisage that such conceptual change would at least simplify the development of computational models.

Community ecology research rarely studies entire communities, in part due to the logistical problems involved in studying multispecies systems, but mostly due to the lack of tools to approach the complex intricacies of ecological communities. The results gathered here are unique as the experimental exclusion of more than one group was performed concomitantly in the field in a self-contained but diverse system, and the densities of almost all guilds were monitored. The challenges of replicating this study in a larger or less self-contained community are evident, but should not be a barrier to further investigation into the generality of the findings presented here. I hope that this study will instigate new methodologies for more holistic approaches to studying ecological communities in general. Developing new methods of applying complexity theory to ecosystems, as suggested above, seems to be one way forward. CAS theory may help elucidate ecosystem structuring and increase our predictability of complex systems.

Figures

Figure 1. Effect network on the host plant *Baccharis dracunculifolia* showing with links categorised into two main types: node modulation (black arrows), which are pairwise trophic (solid arrows) and non-trophic interactions (dashed arrows); and link modulation, which are three-way interactions (interaction modification; dashed red arrows), or four-way interactions (modification of an interaction modification; dashed blue arrows). The link codes (numbers) refer to interactions and corresponding statistical tests depicted in Tables 1 and 2.



Tables

Table 1. Interactions among groups of arthropods on the host plant *Baccharis dracunculifolia*. The link codes refer to interactions depicted in Figure 1, and the relevant statistical tests are presented in Table 2. G7 and G10 refer to the gallers *Baccharopelma dracunculifoliae and Rachiptera limbata*, respectively.

Link code	**Interaction type**	**Affecting**	**Effect**	**Affected**	**Statistics (see Table2)**
1	Node modulation	Galler G7	Negative	Host plant	NA
2	Node modulation	Parasitoid	Negative	Galler G7	NA
3	Node modulation	Galler G10	Negative	Host plant	NA
4	Node modulation	Parasitoid	Negative	Galler G10	NA
5	Node modulation	Ants	Positive	Predator abundance	line 2
			Negative	Predator richness	line 18
			Negative	Predator abundance	line 19
6	Node modulation	Ants	Negative	Aphid (on branches)	line 26
		Aphids (on branches)	negative	Ants	line 27
7	Node modulation	Ants	Negative	Relationship nymph abudance x volume of Gall G7	line 46
8	Link modulation (four-way)	Ants	Positive	Link 30	line 56
9	Link modulation	Ants	positive	Relationship Parasitoid x Gall G7	line 69
10	Link modulation	Ants	Positive	Relationship Galler G10 x Host Plant	line 93
11	Link modulation	Ants	positive	Relationship Aphid x Gall G7	line 91
12	Link modulation (2nd degree)	Ants	positive	Link 13	line 88
13	Link modulation	Aphid (inside galls)	positive	Relationship Galler G10 x Host Plant	line 88
14	Node modulation	Aphid (inside galls)	negative	Galler G7	lines 38, 49, 55
15	Node modulation	Aphid (inside galls)	negative	Parasitoids of galler G7	line 52
16	Link modulation	Aphids (on branches)	Negative	Relationship Parasitoid x Gall G7	line 75
17	Node modulation	Hatched Gall G7	Positive	Ants	line 92
18	Link modulation (2nd degree)	Hatched Gall G7	Positive	Link 30	line 56
19	Link modulation	Hatched Gall G7	Positive	Relationship Parasitoid x Gall G7	line 41
20	Link modulation (2nd degree)	Hatched Gall G7	Positive	Link 9	line 69
21	Link modulation	Hatched Gall G7	Positive	Relationship Aphid x Gall G7	line 90
22	Link modulation	Hatched Gall G7	Positive	Relationship Parasitoid x Galler G10	line 78
23	Link modulation	Hatched Gall G7	Positive	Relationship Galler G10 x Host Plant	line 87
24	Link modulation (2nd degree)	Hatched Gall G7	Positive	Link 10	line 93
25	Node modulation	Gall G7	Positive	Predator abundance	line 2
26	Link modulation	Gall G7	Negative	Relationship Ant presence x Aphid presence (on branches)	lines 26, 27
27	Link modulation	Parasitoid	Positive	Relationship Aphid x Gall G7	lines 31, 35
28	Link modulation	Predator richness	Positive	Relationship Parasitoid x Gall G7	line 56
		Predator abundance	Positive	Relationship Parasitoid x Gall G7	line 59
29	Node modulation	Herbivores	Negative	Host plant	NA

Table 2. Summary of results of statistical tests. The interaction numbers refer to corresponding interactions described in Table 1 and depicted in Figure 1. The values highlighted in bold are statistically significant ($P < 0.05$). G7 and G10 refer to the gallers *Baccharopelma dracunculifoliae and Rachiptera limbata,* respectively.

Line	Dataset	Interaction	Response	Explanatory variable	χ^2	d.f.	p	Post hoc comparison
1	*Observation*		Predator richness	Treatment : Week	26.937	15	**0.029**	In week 6, Hatched < Ant exclusion (Tukey's HSD, p = 0.030)
2		5, 25	Predator abundance	Treatment : Week	40.055	15	**<0.001**	In week 2, Control > Ant exclusion (Tukey's HSD, p = 0.034), and Live gall excluded (p = 0.008)
3			Herbivore richness	Treatment : Week	20.063	15	0.169	
4				Week	4.180	5	0.523	
5				Treatment	8.985	3	**0.029**	Ant exclusion > Hatched gall excluded (Tukey's HSD, p = 0.012)
6			Herbivore abundance	Treatment : Week	18.705	15	0.227	
7				Week	7.364	5	0.194	
8				Treatment	8.380	3	**0.038**	Ant exclusion > Hatched gall excluded (Tukey's HSD p = 0.028)
9			Aphid presence	Treatment : Week	3.291	3	0.348	
10				Week	152.800	1	**<0.001**	
11				Treatment	0.404	3	0.939	
12			Ant richness	Treatment : Week	4.700	2	0.095	
13				Week	45.383	1	**<0.001**	
14				Treatment	0.039	2	0.980	
15			Ant presence	Treatment : Week	5.481	2	0.064	
16				Week	62.948	1	**<0.001**	
17				Treatment	0.321	2	0.851	
18		5	Predator richness	Ant presence : Treatment	8.403	2	**0.014**	t = -2.54; Slopes comparison: Control < Live gall exclusion (p = 0.05)
19		5	Predator abundance	Ant presence : Treatment	14.658	2	**<0.001**	t = -1.963; Slopes comparison: Hatched gall excluded < Live gall exclusion (p < 0.001)

20			Herbivore richness	Ant presence : Treatment	1.724	2	0.422	
21				Ant presence	0.671	1	0.412	
22				Treatment	2.269	2	0.321	
23			Herbivore abundance	Ant presence : Treatment	1.706	2	0.426	
24				Treatment	0.882	2	0.643	
25				Ant presence	0.337	1	0.561	
26		6, 26	Aphid presence	Ant presence : Treatment	21.192	2	**<0.001**	t = -1.480; Slopes comparison: Control < Live gall exclusion (p = 0.022)
27		6, 26	Ant presence	Aphid presence : Treatment	26.030	2	**<0.001**	t = -0.670; Slopes comparison: Control < Live gall exclusion (p < 0.001)
28			Shoot growth	Treatment	0.952	1	0.329	
29	*Dissection*		Aphid per gall	G7 Parasitism : Treatment	3.899	2	0.142	
30				Treatment	3.920	2	0.140	
31		27		Parasitism	19.042	1	**<0.001**	t = 6.005
32			Aphid per gall	G7 Parasitism (binary) : Treatment	2.374	2	0.305	
33				Block	7.948	15	0.925	
34				Treatment	2.173	2	0.337	
35		27		G7 Parasitism (binary)	6.144	1	**0.013**	t = 2.630
36			Aphid per gall	Gall volume: Treatment	1.137	2	0.566	
37				Block	17.703	15	0.278	
38		14		Gall volume	6.602	1	**0.010**	t = -2.586
39				Treatment	7.264	2	**0.026**	
40			G7 Abundance	Treatment	2.351	2	0.308	
41		19	G7 Parasitism	Treatment	7.805	2	**0.020**	Control > Hatched gall exclusion (z = 2.834; df = 1; p = 0.004)
42				Block	32.614	15	**0.005**	
43			G7 volume	G7 Parasitism : Treatment	1.051	2	0.591	

44				Treatment	0.042	2	0.979	
45				G7 Parasitism	0.855	1	0.355	
46		7	G7 volume	G7 nymphs per gall : Treatment	10.317	2	**0.005**	t = 2.061 Slopes comparison: Control < Ant exclusion (p = 0.003)
47			G7 volume	Aphid per gall : Treatment	1.031	2	0.597	
48				Treatment	8.316	2	0.841	
49		14		Aphid per gall	0.345	1	**0.003**	z = -2.872
50			Parasitoid mortality	Inquilinism (binary) : Treatment	4.813	2	0.090	
51				Treatment	0.147	2	0.928	
52		15		Inquilinism (binary)	10.285	1	**0.001**	t = 3.465
53			G7 nymph mortaity	Aphid per gall : Treatment	3.380	1	0.184	
54				Treatment	0.360	2	0.835	
55		14		Aphid per gall	5.924	1	**0.014**	t = 2.520
56	*Observation /Dissection*	8, 18, 28	G7 Parasitism	Predator richness : Treatment	8.878	2	**0.011**	z = 2.819; Slopes comparison: Control > Ant excluded (z = -2.677; df = 1; p = 0.007), and Hatched gall excluded (z = -2.677; df = 1; p = 0.016)
57			G7 Parasitism	Predator abundance : Treatment	3.878	2	0.143	
58				Treatment	10.420	2	**0.005**	
59		28		Predator abundace	21.054	1	**<0.001**	z = 0.481; df = 1; p = <0.001
60			G7 Parasitism	Herbivore richness : Treatment	0.693	2	0.071	
61				Herbivore richness	1.837	1	0.175	
62				Treatment	6.964	2	**0.030**	
63			G7 Parasitism	Herbivore abundance : Treatment	5.834	2	0.054	
64				Herbivore richness	0.692	1	0.405	
65				Treatment	5.362	2	0.068	
66			G7 Parasitism	Ant richness: Treatment	0.088	1	0.766	
67				Ant richness	0.100	1	0.750	

68				Treatment	5.008	1	**0.025**	
69		9, 20	G7 Parasitism	Ant presence : Treatment	8.137	1	**0.004**	z = 2.687; Slopes comparison: Control > Hatched excluded (z = -2.800; df = 1; p = 0.005)
70			G7 Parasitism	Hatched abundance : Treatment	0.018	1	0.893	
71				Treatment	0.986	1	0.320	
72				Hatched abundance	2.376	2	0.123	
73			G7 Parasitism	Aphid presence : Treatment	5.601	2	0.060	
74				Treatment	5.048	2	0.080	
75		16		Aphid presence	8.014	1	**0.004**	z = -2.811; df = 1; p = <0.004
76			G10 parasitism	Hatched abundance : Treatment	1.218	2	0.543	
77				Treatment	1.880	2	0.390	
78		22		Hatched abundance	4.768	1	**0.028**	z = 2.085; df = 1; p = 0.037
79			G10 parasitism	Aphid presence : Treatment	0.200	2	0.904	
80				Aphid presence	0.606	1	0.436	
81				Treatment	1.880	2	0.390	
82			G10 parasitism	Ant presence : Treatment	0.231	1	0.630	
83				Ant presence	0.182	1	0.668	
84				Treatment	1.415	1	0.234	
85			G10 abundance	Hatched abundance : Treatment	0.902	1	0.342	
86				Treatment	0.304	1	0.581	
87		23		Hatched abundance	4.134	1	**0.042**	z = -2.714, df = 1; p = 0.042
88		12, 13	G10 abundance	Aphid per gall : Treatment	8.621	2	**0.013**	t = 1.327; Slopes comparison: Ant exclusion < Control (p = 0.034)
89	*Simple Linear Models*				**R**	**F**	**p**	
90		21	Aphid per gall	Hatched abundance	0.363	7.139	**0.003**	t = 3.079, df = 1, p = 0.004

91	11		Treatment	0.363	7.139	**0.003**	Ant exclusion < Control (t = - 2.387, df = 1; p = 0.024)
92	18	Ant presence	Hatched abundance	0.177	4.025	**0.044**	t = 2.006
93	10, 24	G10 abundance	Ant presence : Treatment	0.228	2.66	**0.026**	t = 2.756, p = 0.010; Slopes comparison: Hatched exclusion < Control (t = -2.150; df=1, p = 0.040)
94		G10 abundance	Ant richness : Treatment	NS			
95		Aphid presence	Hatched abundance : Treatment	NS		0.459	
96			Treatment	NS		0.873	
97			Hatched abundance	NS		0.722	

GENERAL DISCUSSION

GENERAL DISCUSSION

The present work aimed to investigate the role of trophic and non-trophic indirect interactions in structuring communities. I experimentally manipulated species densities in a diverse and self-contained assemblage of arthropods living on a shrub species in a Brazilian savannah habitat. In distinct experiments, I either reduced a species abundance, completely excluded a species, or limited a species byproduct. Then I quantified the density of the other species, as well as the strength of their interactions, to explore ways in which individual species can, directly and indirectly, affect the structure and stability of a relatively simple and discrete ecological community.

In the **first chapter**, I investigated the role of direct and indirect interactions on food web structure and robustness (tolerance to species loss). I experimentally reduced the abundance of the most common gall-making insect species found on the host plant and found that the perturbation resonated across the entire food web, affecting the food web structure and robustness. Since there was no potential for these effects to be propagated directly or indirectly via the documented trophic interactions, the effects must have spread non-trophically and/or through trophic links not included in the webs. The results highlight that both trophic and non-trophic direct and indirect interactions are essential to understanding the structure and dynamics (Fontaine et al. 2011, Kéfi et al. 2012) of even apparently discrete ecological communities.

In **chapter two**, I examined the non-trophic propagation of effects in the same system. Aphids occupy live galls of the commonest galler as well as hatched ones, which dry out and remain attached to the plant. I hypothesised that the hatched galls could generate feedbacks on the galler – e.g., increasing the availability of shelter for aphids and therefore positively affecting the galler by decreasing aphid occupation of live galls. Thus, I excluded or added hatched galls to plants in different patches. The results show that hatched galls of the dominant galler can trigger non-trophic interactions that feedback on the galler modifying its interactions with both parasitoids and inquiline aphids. In addition, the results suggest that those interactions are dependent on the context, which changes with species densities. This work is one of the first to empirically demonstrate the context-dependent modification of interactions via non-trophic interactions.

In **chapter three**, I investigated whether the non-trophic interaction regulation observed in the second chapter are common amongst other species living on the same plant. I looked at how the abundances of certain pairs of groups relate to each other, and how their relationship changes in the presence or absence of a third group. I identified many other non-trophic indirect interactions regulating interactions. The work reveals that non-trophic modification of interaction is common among distinct sets of species in this system, and is therefore likely to be widespread in nature.

Lastly, in **chapter four**, using the dataset from chapter three, I built an "effect network" portraying trophic and non-trophic interactions. It revealed a hidden web of indirect effects modifying the direct interactions and modifying each other. The removal of different groups seemed to indirectly affect the relationships between several other pairs of groups to which the manipulated groups were not directly related. This could be one of the first truly empirical studies to demonstrate, for an entire community, that most, if not all, direct interactions are modulated by many concurrent indirect interactions.

In sum, the thesis presents evidence that communities are strongly interconnected through indirect interactions, with components exerting global effects not limited to their predators or prey. In addition, the direct interactions are dependent on the context of indirect interactions modifying them, which changes with species density. As a result, the direct effect of one species on another represented as a defined direct link would be an abstraction, as in reality that effect is dependent on the whole context of a web of indirect effects modulating them.

Throughout this thesis two main themes are present, the mechanisms behind the non-trophic interactions, and their role in community stability. I discuss these themes in turn, then suggest future research directions leading on from my thesis.

Mechanisms of non-trophic interactions

Communities are essentially interacting species, and species can interact in many different ways. Ecologists have therefore used networks of nodes (species) and links (interactions) to represent community structure. Initially, representing the feeding relationships (trophic links) seemed important and perhaps more intuitive as those are the most visible interactions in an ecological community. Then, it became evident the need to incorporate less obvious interactions in that representation, including indirect links, that is, interactions that have a third species as a mediator (e.g., apparent competition; Holt 1977). It is also clear that interactions not involving feeding relationship (non-trophic interactions - NTI) are as important, or even more important, than trophic interactions, and therefore should also figure in networks representing communities (Kéfi et al. 2012, Pocock et al. 2012). Nonetheless, NTI are much less consistent and “visible”, and therefore difficult to be integrated into network theory.

The difficulty lies mostly in the fact that, unlike direct interactions, which are relatively easy to record (e.g., predation, parasitism), indirect interactions can only be described in terms of their mechanisms, that is, how they are mediated. The outcome alone is not enough to determine whether the interaction is actually taking place.

Unveiling the mechanism of an indirect interaction is complicated by the fact that a mechanism implies a fixed rule. NTI defies mechanistic explanations due to the fact that they often interfere with and modify each other in a non-linear and transient way. For instance, it is possible to use equations to describe a predator-prey interaction, or even how two preys indirectly affect each other through a shared predator. However, as the present study shows, when it comes to indirect NTI, the possibilities of pathways in a network seem endless and pathways become more complex, often involving more than one intermediate. In addition, unlike trophic interactions, some NTI only seem to occur in certain contexts defined by species abundances. For example, the galler-parasitoid interaction in the focal study system in this thesis is permanent, even though its strength may change. However, the non-trophic modification of galler-parasitoid interaction by aphid inquilines only occurred at higher gall abundance. As a result, whilst it is relatively easy to identify an NTI using experiments similar to the ones reported here, determining a definite pathway for the interactions has proven much more difficult. Even if we are able to experimentally establish an NTI pathway, there is no evidence whether or not that pathway is permanent or transitory, or how it actually takes place under natural conditions.

The role of NTI in community stability and persistence

The fact that a product of ecosystem engineering, such as a hatched gall, can play a role in modulating interactions (chapter 2) suggests that indirect effects may be far more widely spread and influential than is realised. Every species has the potential to trigger NTI, whether via engineering or other countless means (e.g., facilitation, interference competition, trait and behaviour modification). This introduces the possibility that a complex network of NTI, like the one shown in chapter 4, could be constantly modifying interactions and modulating ecological communities.

NTI are conditional on the presence and density of other species (context-dependent), which furthermore varies in time. Since NTI modify pairwise interactions, in nature, pairwise interactions are also non-linear. Consequently, pairwise interactions taken separately, such as in a web link, are abstractions, as they only exist in the context of a web of indirect effects modulating them. For example, the interaction between species A and B that would be linear if taken separately will be non-linear in nature. This is because, through indirect effects, species A may, for instance, benefit a competitor, host, or prey, or affect an environmental condition that will in turn affect species B, perhaps changing the overall effect of A on B. That could be why, for instance, an effect of an experimental manipulation, or an interaction is sometimes evident and other times undetectable. It is difficult to understand how one species affects another, when the end effect is a combination of several influences from many additional species.

If a complex network of NTI is constantly modifying interactions in ecological communities, non-trophic modification of interactions may be key to species coexistence, and community persistence and dynamic stability (Golubski & Abrams 2011, Hammill et al. 2015, Kéfi et al. 2015, Benson et al. 2016). Here, the effects of manipulating single groups on population densities were never very obvious. It was only by focusing on particular interactions that I found that species were indirectly affected by the experimental manipulations. Although I have no empirical evidence showing it, in chapter 2, I argued that the system may have a mechanism to keep populations relatively stable despite disturbances as a result of changes in the magnitude of interactions. If there is such an internal mechanism of community stability, NTI is likely to play an important role in it. First, NTI seem to be widespread and can influence several groups cementing the assemblages. Second, they are context-dependent and regulated by feedbacks, which means that they will change their influence on interactions according to population densities (see chapter 2). Thus, further investigation of such principles of modulation, using computational analyses, for instance, could help unveil the mechanistic basis of community structuring and stability.

Future directions

The challenges of defining or describing NTI are of such magnitude that it may lead us to question whether we should move away from using networks to represent communities altogether. Ecological pairwise interaction networks seem inadequate to depict a species' role in a community unless they account for the context-dependent non-trophic modification of interactions. The results here confirm that integrating NTI to ecological networks, as suggested by recent works (Fontaine et al. 2011, Kéfi et al. 2012), is essential. However, this would only be possible if we were able to account for the interference among effects, which causes non-linearity and makes interactions circumstantial. For instance, in a multiplex network, it is possible to map positive and negative NTI onto a layer of trophic links (Kéfi et al. 2016). However, this approach is still incomplete as it leaves out non-trophic modification of non-trophic interactions (four-way interactions). The greatest challenge would be to map the NTI onto each other, to account for interactions among them modifying each other. This could be done similarly to what is presented in chapter four here, but ideally with more quantitative information on the strength of the effects.

Whether or not it is possible to map a network including all the possible NTI, the question comes down to whether it is valid to study abstracted parts of the community that we are able to represent using networks. In nature these abstract interactions would be embedded in a web of NTI interfering with or modifying them and not isolated. It clearly depends on the questions that we are addressing. Undoubtedly, community ecology theory through network approaches has made huge progress with understanding the structure of communities (e.g., Lewis et al 2001, 2002); and with related practical applications – e.g.investigating infiltration of alien species (Henneman & Memmott 2001). However, to what extent is that community ecology representative of the "real world"? One could argue that the questions that community ecologists have been asking are the questions that we can address with current network theory, and not necessarily the most relevant ones. Or, that the answers that we get are limited to a set of conditions and assumptions and lack general explanatory value. Perhaps we should be addressing more fundamental questions, such as what internal dynamics lead to the stability of whole communities? Or how do simple assembly rules lead to complex structures?

I propose that rather than focusing on the outcome of specific pairwise interactions, for example, how parasitoids affect the dynamics of their hosts, we should take a community or system based approach (Fontaine et al. 2011, Wooton 1994, Kéfi et al. 2012). This would allow us to recognise fundamental mechanisms involving NTI modulation that, for instance, allow the system (or community) as a whole to adapt to a specific manipulation such as the removal of a galler species. Advancing our understanding of such mechanisms is likely to elucidate the underlying causes of stability and persistence of ecological communities and increase our ability to predict how they might respond to perturbations. Computational simulations that account for non-trophic indirect effect will be helpful, but the results presented here suggest that empirical data will be key to our understanding because of the numerous and often unpredictable opportunities for indirect effects via a variety of mechanisms and pathways.

Conclusion

This thesis presents evidence that communities are strongly interconnected through non-trophic indirect interactions. Thus, trophic and non-trophic direct and indirect interactions are essential to understanding the structure and dynamics (Fontaine et al. 2011, Kéfi et al. 2012) of even simple ecological communities. This study is unprecedented in terms of empirical demonstration of the mechanisms behind non-trophic interactions, and their role in community stability at the community level. One of the reasons for the lack of studies similar to this is that detecting and measuring indirect interactions in natural communities is an enormous challenge (Wootton 1994, Abrams 2008; Novak & Wootton 2008). The results reveal and illustrate the context-dependent modification of interactions via non-trophic interactions; and that most, if not all, direct interactions are modulated by many concurrent indirect interactions. However, the study also demonstrates that determining the exact mechanisms behind such interaction modifications may be unfeasible because so many different pathways may be involved. Understanding how the observed effects relate to community structuring requires shifting our focus from bipartite interaction networks to a more holistic approach.

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