Experimental evidence for a hidden network of higher-order interactions in a diverse arthropod community

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**Summary**

Transcending pairwise interactions in ecological networks remains a challenge 1–5. Higher-order interactions (HOIs), the modulation of a pairwise interaction by a third species 6, are thought to play a particularly important role in stabilising coexistence and maintaining species diversity 7–12. However, HOIs have so far only been demonstrated in models 9–14 or isolated experimental systems including only a few interacting species 7,8,15. Their ubiquity and importance at a community level in the real world remain unknown. We hypothesised that a complex network of HOIs could be constantly modifying pairwise interactions and shaping ecological communities, and that consequently the outcome of pairwise interactions would be a product of many influences from distinct sources. Using field experiments, we tested how multiple interactions within a diverse arthropod community associated with the tropical shrub *Baccharis dracunculifolia* D.C. (Asteraceae) were modified by the removal of ant species or live or hatched insect galls (a non-trophic engineering effect) of the dominant galler species. We revealed an extensive hidden network of HOIs modifying each other and the “visible” pairwise interactions (Figure 2). Most pairwise interactions were affected indirectly by the manipulation of non-interacting taxonomic groups. The pervasiveness of these interaction modifications challenges pairwise approaches to understanding interaction outcomes and could shift our thinking about the structure and persistence of ecological communities. Investigating coexistence mechanisms involving interaction modulation by HOIs may be key to elucidating the underlying causes of the stability and persistence of ecological communities.

**Key words:** ants**;** aphids; *Baccharis dracunculifolia*; Cerrado; experimental manipulation; galling insects; indirect interactions; interaction modification; parasitoid wasps; parasitism

**Results**

We predicted that the manipulation of any species (or group of related species) within the community - or even of one of their non-trophic engineering effects (e.g. hatched galls) - would reverberate throughout the entire community and thus modify apparently unrelated interactions. The shrub *B. dracunculifolia* is a self-contained system with a diverse arthropod fauna located in the Brazilian Cerrado (see STAR Methods). The system includes 15 ant species, nine predator species (mostly spiders and ladybirds), 41 free‐feeding herbivorous insect species, 17 galler species (organisms, in this case insects, which induce an abnormal growth, or gall, on the plant, and develop inside it), and about 50 parasitoid wasps attacking the gallers.16 The focal galler, *Baccharopelma dracunculifoliae* (Sternorrhyncha: Psyllidae), is the most abundant and can be viewed as an allogenic ecosystem engineer (acting on other living or non-living structures) by inducing galls.15 The galls remain attached to the plant for a few months after the emergence of the galler and gradually become dry and woody. Both live and hatched galls of *B. dracunculifoliae* are occupied by many invertebrates, such as ants, spiders and aphids,17 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; it lives on terminal buds but also colonises fully developed galls and can lead to the death of galler larvae.18,19

We created four treatments: (i) *Ant Exclusion* – all ants were excluded by applying a non-toxic resin to the basal stem of the plants; (ii) *Live Gall Exclusion* – *Baccharopelma dracunculifoliae* (Sternorrhyncha: Psyllidae),the commonest galling species found on *B. dracunculifolia* was removed from the plantsby hand collecting the galls; (iii) *Hatched Gall Exclusion* - All hatched galls (a non-trophic engineering effect) of *B. dracunculifoliae* were removed by hand collecting the galls; and (iv) *Control* - no exclusion. Each treatment consisted of 16 replicated plants of *B. dracunculifolia*. Over two months, every week we quantified the changes in densities of several other species or guilds (hereafter groups; ants, free-feeding herbivores, predators, and aphids) as well as changes in the direct interactions of two galler species, such as gall induction (herbivory), parasitism by wasps, and inquilinism by aphids. Specifically, we combined direct observation and gall dissection data to quantify the effects of groups on each other’s population densities or traits (gall volume and plant size), including direct trophic and non-trophic interactions and the effect of the exclusion of a group on another group (hereafter, density effects; Figure 1). We also investigated how the direct interactions changed in different contexts defined by the density or exclusion of a third group (see STAR Methods). This allowed the construction of a unique “effect network” based on multiple manipulations performed simultaneously on the same system. Links were categorised into two types: node modulation (node-to-node effects), which are pairwise trophic and non-trophic interactions or density effects; and link modulation (HOIs; node-to-link effects), which are three-way interactions (interaction modification), or four-way interactions (modification of an interaction modification). Based on our previous studies manipulating the same system15,16 we expected small effects overall as a result of removal treatments, because many, potentially opposing, effects will be taking place concurrently.

Over two months we quantified 1,427 ants from 15 different species found on 988 branches, 1,109 predators from nine morphospecies (spider species were grouped as a single morphospecies), 629 free-feeding herbivore insects from 41 morphospecies, and 22,564 terminal buds occupied by aphids. In the last week of the experimental period, we quantified 365 live galls (Mean = M; M = 22.812; Min = 0; Max = 64) and 559 hatched galls (M = 34.937; Min = 0; Max = 130) of *Baccharopelma dracunculifoliae* (Sternorrhyncha: Psyllidae) in the Control treatment; 474 live galls (M = 29.625; Min = 4; Max = 100) and 723 hatched galls (M = 45.187; Min = 0; Max = 125) in the Ant Exclusion treatment; and 421 live galls (M = 26.312; Min = 0; Max = 74) in the Hatched Gall Exclusion treatment. A total of 522 live galls of *B. dracunculifoliae* were collected and dissected. We also quantified and dissected 34 live galls (M = 2.125; Min = 0; Max = 11) of the galler *Rachiptera limbata* Bigot (Diptera: Tephritidae) from the Control treatment; 30 (M = 1.875; Min = 0; Max = 9) from the Ant Exclusion treatment; and 29 (M = 1.812; Min = 0; Max = 6) from the Hatched Gall Exclusion treatment. The effects identified are portrayed in an effect network with ten nodes (two galling species and their respective parasitoid wasps, hatched galls, ants, aphids, herbivores, predators and the host plant) and 29 links (Table 1, Figure 2). There were ten node modulation links - five trophic and two non-trophic direct interactions, and three density effects; and 19 link modulation links - 12 three-way links, and five four-way links.For the sake of simplicity, the results of the statistical tests for each link in the network are shown in Table 2 (see also Figures S1 – S4). Ants modulated two density effects and four three-way interactions; live galls of *B. dracunculifoliae* modulated one density effect, one pairwise and one four-way interaction; and hatched galls of *B. dracunculifoliae* modulatedfour three-way and four four-way interactions (Table 1, Figure 2 and S1).

Excluding ants reduced the abundance of predators (link 9 - all link codes hereafter refer to Table 1 and Figure 2). Also, in the Ant Exclusion treatment, the positive relationship between the number of galling nymphs and volume of *B. dracunculifoliae* galls was stronger (link 8). Ant frequency on branches was positively correlated with parasitism of the galler *B. dracunculifoliae* (link 12), as well as with the abundance of the galler *R. limbata* on the plants (link 13), and negatively related to the frequency of aphids on branches (link 14). Ant exclusion lowered the number of inquiline aphids inside *B. dracunculifoliae* galls (link 11) - ants seem to facilitate the aphid-galler interaction, apparently by leading or even carrying aphids inside galls.

Excluding the galler *B. dracunculifoliae* reduced the abundance of predators, but only in the second week of the two-month study period (link 10); it also positively affected (link 25) the relationship between ant frequency and aphid frequency on branches (link 14) over the two months.

Excluding hatched galls reduced parasitism of the galler *B. dracunculifoliae* (link 16). Plants with higher abundance of hatched galls also presented higher aphid inquilinism (link 17), higher abundance of the galler *R. limbata* (link 18), and higher parasitism of the galler *R. limbata* (link 19). In the Hatched Gall Exclusion treatment, the positive relationship between ant frequency and the abundance of the galler *R. limbata* (link 13) was weakened (link 26). Excluding hatched galls also weakened (link 27) the positive relationship between ant frequency and parasitism of the galler *B. dracunculifoliae* (link 12); weakened (link 28) the negative relationship between herbivore abundance and the abundance of the galler *B. dracunculifoliae* (link 20); and also weakened (link 29) the positive relationship between predator abundance and aphid inquilinism (link 23).

Galls of *B. dracunculifoliae* with higher aphid inquilinism presented lower volume, and higher nymph mortality (link 1). Mortality of parasitised nymphs was also higher in galls with more aphid inquilines (link 2). On plants with more branches occupied by aphids, parasitism of the galler *B. dracunculifoliae* was lower (link 15).Aphid inquilinism (link 1) was higher in parasitised galls of the galler *B. dracunculifoliae* (link 21). Parasitoids appeared to facilitate the interaction between the aphid inquilines and the galler *B. dracunculifoliae*, possibly by changing gall shape and allowing aphids to enter through the longitudinal aperture of the gall.

We identified several three-way and four-way interactions taking place concurrently, demonstrating how multiple (and at times contrasting) effects combine to create overall effects on species densities. For example, inquiline aphids can kill the nymphs of the galler *B. dracunculifoliae* (link 1), but because they preferentially occupy parasitised galls (link 21) and can kill parasitised nymphs (link 2), they can negatively affect parasitism and therefore also benefit the galler *B. dracunculifoliae* (link 15). As another example, aphid-tending ants, by increasing aphid inquilinism (link 11), possibly by leading aphids into galls, may positively affect parasitism on the galler *B. dracunculifoliae* (link 12) by reducing aphids on branches (links 14) and therefore their negative effect on parasitism (link 15). On the other hand, by increasing aphid inquilinism, ants negatively affect parasitoids, since parasitoid mortality is higher in galls occupied by aphids (link 2). Furthermore, hatched galls increase parasitism on the galler *B. dracunculifoliae* (link 16), perhaps serving as a cue for parasitoids, but they also increase aphid inquilinism (link 17) and therefore can reduce parasitism on the galler *B. dracunculifoliae*. Finally, parasitism of the galler *B. dracunculifoliae* was also positively associated with ant frequency on branches (link 12), but the relationship was dependent on the presence of hatched galls (link 27; four-way interaction).

Every direct interaction on the network was influenced by at least one of the other groups. In several cases, it was not possible to propose a mechanism behind the indirect links, even though the natural history of the system is fairly well-known.

**Discussion**

Whilst we already knew from studies on subsets of communities that non-trophic indirect effects must be important for understanding community structure and dynamics 1–3,6,7,15,20–22 this is thought to be the first truly empirical study to demonstrate, for an entire diverse community in the field, how numerous HOIs are acting at the same time. Here, we not only explored community-wide effects but experimentally tested each of the interaction modifications identified in situ. By performing several manipulations concomitantly under natural field conditions, we were able to study the same interactions in different contexts and detect how multiple non-trophic interactions can interfere with or modify a single pairwise interaction. Thus, HOIs are shown not to be particular to certain sets of species but rather an integral part of communities. The manipulations revealed a hidden network of HOIs modifying the direct interactions, as well as modifying each other, a level of complexity unexplored in empirical studies and seldom mentioned in theoretical studies.9,10,12,13 Whilst our study system focused on an insect community on a tropical shrub, a hidden network of HOIs will occur in all ecological communities, and therefore our results are of huge ecological relevance. The removal of different groups indirectly affected the interactions between several other pairs of groups with which the manipulated groups did not directly interact. That indicated that the species are indirectly connected and pairwise interactions are context-dependent. We acknowledge that the three density effect links in the network may represent species association and not true links (it would be difficult to differentiate between the two), which would mean that the groups do not actually affect one another directly and may be both affected by a third group. However, we include these links, first, because it would be difficult to clarify whether that is the case and, second, because the fact that a node changes in the absence of other groups shows that they are somehow connected, therefore there is potential for indirect interaction, albeit via additional intermediate groups. Also, the HOIs are represented by straight arrows in the network, but in reality, multiple steps may be involved in their effect propagation pathways. What the arrow represents is that the pairwise interaction is affected by the third group. This level of detail on indirect non-trophic interactions, and in particular for HOIs, for such a species-rich multi-trophic system, is truly unsurpassed.

It seems almost impossible to determine how a species affects another when we consider that all those HOIs may be taking place at the same time and interfering with each other. Besides, the links represented in the network may vary in magnitude through time, or may even be transient 15. In the community studied, population densities at a given time are 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 in an experimental setting or in computational models. Thus, depicting direct links between species in a network may be a misrepresentation of species effects or roles at the community level.

The existence of this hidden network suggests that, in natural conditions, it is unlikely that one species alone can determine the persistence of any other, such as in competitive exclusion. The role of competition in species coexistence has been widely demonstrated for focal species 23,24, but rarely in species-rich communities 25–27. Here we show that HOIs can hugely increase the context-dependency of pairwise interactions, and by modifying interactions and offsetting or complementing each other, can allow a flexible modulation of species coexistence. Thus, the hidden network of HOIs very likely plays a crucial role in diversity maintenance in multispecies communities.

It is imperative to devise methods to harmonize pairwise interaction networks with the hidden network of HOIs. This would allow us to recognise fundamental mechanisms involving interaction modulation by HOIs that, for instance, allow the community as a whole to respond to a specific manipulation such as the removal of a species. Advancing our understanding of such mechanisms is likely to elucidate the underlying causes of stability and persistence of ecological communities 7–12 and increase our ability to predict how they might respond to perturbations. Computational simulations that account for HOIs are 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. The challenges of replicating this study in a larger or less self-contained community are evident, but should not be a barrier to further empirical investigations into the generality of the findings presented here.

We hope that this study will instigate new methodologies for more holistic approaches to studying ecological communities because the HOIs identified here could not be predicted from analyses of pairwise networks, or based on species functional traits. Through HOIs, species can have global effects on the community. At the same time, these effects can be transient, varying in intensity and direction depending on the community context (species composition and abundances mainly). This circular relationship between the structure of the global system and the local interactions among the components is typical of complex adaptive systems 28. Thus, developing new methods of applying complexity theory to ecosystems 28 could be one way forward.

**Acknowledgements:** We thank Chris Terry for helpful discussions and acknowledge the logistical support from Reserva Vellozia and Federal University of Minas Gerais. MB was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) grant BEX 1129120, RJM by a Royal Society University Research Fellowship, and GWF by the Brazilian National Council for Scientific and Technological Development (CNPq) grant 316258/2021-0 and by the Minas Gerais State Agency for Research and Development (Fapemig).

**Author contributions:** MB and RJM designed the experiment, MB collected the data with assistance from GWF, MB analysed the data with support from RJM; MB wrote the first draft of the manuscript and all authors edited the manuscript and contributed substantially to the ideas presented.

**Declaration of interests:** The authors declare no competing interests.

**Figure 1. Methods used to detect and quantify different effects in the network:** (A) Node modulation effects: (i) pairwise trophic and non-trophic interactions were measured through gall dissection or direct observation, and (ii) density effects were effects on a node’s density or trait (gall volume and plant size) following the exclusion of another node; (B) Link modulation effects: (iii) three-way interactions were measured by how direct interactions changed in different contexts defined by the exclusion or density of a third node; and (iv) four-way interactions were measured by how the dependence of an interaction on the densities of other nodes changes in each context of exclusion of a fourth node (see also Tables S1 and S2).

**Figure 2. Effect network on the host plant *Baccharis dracunculifolia* showing links categorised into two main types**: node modulation (black arrows), which are pairwise trophic and non-trophic interactions (solid arrows) or density effects (dashed arrows); and link modulation (higher-order interaction), which are three-way interactions (interaction modification; dashed red arrows), or four-way interactions (modification of an interaction modification; dashed blue arrows). The manipulated nodes are highlighted and crossed through in red. The link codes (numbers) refer to interactions and corresponding statistical tests depicted in Tables 1 and 2 (see also Figures S1 – S4). For node modulation links, the sign means a positive or negative effect on the abundance, survival or trait value (gall volume and plant size) of the receiving interaction partner, while for link modulation it means strengthening or weakening a link.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Link**  **code** | **Link type** | **Affecting** | **Effecta** | **Affected** | **Detection**  **method** | **Statistics (see Table 2)** |
|
| ***Node modulation*** | |  |  |  |  |  |
| 1 | Pairwise interaction | Aphid (inside galls) | Negative | Galler G7 | Gall dissection | lines 7, 8 |
| 2 | Pairwise interaction | Aphid (inside galls) | Negative | Parasitoids of galler G7 | Gall dissection | line 9 |
| 3 | Pairwise interaction | Aphids (on branches) | Negative | Host plant | Observation | NA (herbivory) |
| 4 | Pairwise interaction | Galler G7 | Negative | Host plant | Gall counting | NA (herbivory) |
| 5 | Pairwise interaction | Galler G10 | Negative | Host plant | Gall counting | NA (herbivory) |
| 6 | Pairwise interaction | Parasitoid | Negative | Galler G7 | Gall dissection | NA (parasitism) |
| 7 | Pairwise interaction | Parasitoid | Negative | Galler G10 | Gall dissection | NA (parasitism) |
| 8 | Density effect | Ants (exclusion) | Negative | Volume of Gall G7 x Nymph abundance | Node removal | line 6 |
| 9 | Density effect | Ants (exclusion) | Positive | Predator abundance | Node removal | line 2 |
| 10 | Density effect | Galler G7 (exclusion) | Positive | Predator abundance | Node removal | line 2 |
| ***Link modulation*** | |  |  |  |  |  |
| 11 | Three-way interaction | Ants (exclusion) | Positive | Link Aphid x Galler G7 | Modifier removal | line 4 |
| 12 | Three-way interaction | Ants (on branches) | Positive | Link Parasitoid x Galler G7 | Modifier density | line 13 |
| 13 | Three-way interaction | Ants (on branches) | Positive | Link Galler G10 x Host Plant | Modifier density | line 16 |
| 14 | Three-way interaction | Ants (on branches) | Negative | Link Aphid x Host Plant | Modifier density | line 1 |
| 15 | Three-way interaction | Aphids (on branches) | Negative | Link Parasitoid x Galler G7 | Modifier density | line 15 |
| 16 | Three-way interaction | Hatched Gall G7 | Positive | Link Parasitoid x Galler G7 | Modifier removal | line 5 |
| 17 | Three-way interaction | Hatched Gall G7 | Positive | Link Aphid x Galler G7 | Modifier density | line 11 |
| 18 | Three-way interaction | Hatched Gall G7 | Negative | Link Galler G10 x Host Plant | Modifier density | line 17 |
| 19 | Three-way interaction | Hatched Gall G7 | Positive | Link Parasitoid x Galler G10 | Modifier density | line 19 |
| 20 | Three-way interaction | Herbivore abundance | Negative | Link Galler G7 x Host Plant | Modifier density | line 12 |
| 21 | Three-way interaction | Parasitoid | Positive | Link Aphid x Galler G7 | Gall dissection | line 3 |
| 22 | Three-way interaction | Predator abundance | Positive | Link Parasitoid x Galler G7 | Modifier density | line 14 |
| 23 | Three-way interaction | Predator abundance | Positive | Link Aphid x Galler G7 | Modifier density | line 10 |
| 24 | Three-way interaction | Predator abundance | Positive | Link Galler G10 x Host Plant | Modifier density | line 18 |
| 25 | Four-way interaction | Galler G7 | Positive | Link 14 | Modifier removal | line 1 |
| 26 | Four-way interaction | Hatched Gall G7 | Positive | Link 13 | Modifier removal | line 16 |
| 27 | Four-way interaction | Hatched Gall G7 | Positive | Link 12 | Modifier removal | line 13 |
| 28 | Four-way interaction | Hatched Gall G7 | Positive | Link 20 | Modifier removal | line 12 |
| 29 | Four-way interaction | Hatched Gall G7 | Positive | Link 23 | Modifier removal | line 10 |
| aPositive and negative modulation respectively strengthens and weakens a link. | | | | | | |

**Table 1. Interactions among groups of arthropods on the host plant *Baccharis dracunculifolia****.* The link codes refer to interactions depicted in Figure 2, and the relevant statistical tests are presented in Table 2 (see also Figures S1 – S4). G7 and G10 refer to the gallers *Baccharopelma dracunculifoliae and* *Rachiptera limbata,* respectively. For example, in figure 2, link three (aphid herbivory) is shown to be modified by link 14 (ants weaken the interaction) and the related statistical result for link modulation 14 is reported in line 1 of Table 2. NA stands for not applicable statistical test.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Line** | **Dataset** | **Interaction** | **Response variable** | **Explanatory variable** | **χ2** | **d.f.** | **p** | **Score / Post hoc comparison** |
| 1 | *Observation* | 14, 25 | Aphid frequency | Ant frequency : Treatment (except Ant exclusion) | 22.454 | 2 | <0.001 | t = -1.745; Slopes comparison: Control < Live gall exclusion (χ2(1) = 7.844, p = 0.010) |
| 2 |  | 9, 10 | Predator abundance | Treatment : Week | 40.055 | 15 | <0.001 | In week 2, Control > Ant exclusion (χ2(1) = 11.823, p = 0.020), and Live gall exclusion (χ2(1) = 14.744, p = 0.008) |
| 3 | *Dissection* | 21 | Inquilinism (aphid/gall) | G7 Parasitism (binary) | 4.897 | 1 | 0.026 | t = 2.325 |
| 4 |  | 11 |  | Treatment (except Live gall exclusion) | 7.157 | 2 | 0.027 | Control > Ant exclusion (χ2(1) = 7.526, p = 0.018) |
| 5 |  | 16 | G7 Parasitism | Treatment (except Live gall exclusion) | 8.175 | 2 | 0.016 | Control > Hatched gall exclusion (χ2(1) = 7.972, p = 0.014) |
| 6 |  | 8 | G7 volume | G7 nymphs per gall : Treatment (except Live gall exclusion) | 6.595 | 2 | 0.036 | t = 2.196; Slopes comparison: Control < Ant exclusion (χ2(1) = 6.635, p = 0.029) |
| 7 |  | 1 |  | Inquilinism (aphid/gall) | 8.162 | 1 | 0.004 | t = -2.872 |
| 8 |  | 1 | G7 nymph mortality | Inquilinism (aphid/gall) | 9.752 | 1 | 0.001 | z = 2.919 |
| 9 |  | 2 | Parasitoid mortality | Inquilinism (aphid/gall) | 33.375 | 1 | <0.001 | z = 2.041 |
| 10 | *Observation /Dissection* | 23, 29 | Inquilinism (aphid/gall) | Predator abundance : Treatment (except Live gall exclusion) | 6.729 | 2 | 0.034 | t = 2.402; Slopes comparison: Control > Hatched gall exclusion (χ2(1) = 6.220, p = 0.037) |
| 11 |  | 17 |  | Hatched abundance | 4.963 | 1 | 0.025 | t = 1.255 |
| 12 |  | 20, 28 | G7 abundance | Herbivore abundance : Treatment (except Live gall exclusion) | 10.215 | 2 | 0.006 | t = -2.604; Slopes comparison: Control < Hatched gall exclusion (χ2(1) = 6.924, p = 0.025) |
| 13 |  | 12, 27 | G7 Parasitism | Ant frequency : Treatment (except Live gall and Ant exclusion) | 8.137 | 1 | 0.004 | z = 2.687; Slopes comparison: Control > Hatched gall exclusion (χ2(1) = 7.840, p = 0.005) |
| 14 |  | 22 |  | Predator abundance | 21.054 | 1 | <0.001 | z = 4.376 |
| 15 |  | 15 |  | Aphid frequency | 8.014 | 1 | 0.004 | z = -2.811 |
| 16 |  | 13, 26 | G10 abundance | Ant frequency : Treatment (except Live gall and Ant exclusion) | 4.770 | 1 | 0.028 | t = 2.668; Slopes comparison: Control > Hatched gall exclusion (χ2(1) = 4.654, p = 0.030) |
| 17 |  | 18 |  | Hatched abundance | 4.134 | 1 | 0.042 | t = -2.714 |
| 18 |  | 24 |  | Predator abundance | 5.234 | 1 | 0.022 | t = 2.445 |
| 19 |  | 19 | G10 parasitism | Hatched abundance | 4.768 | 1 | 0.028 | z = 2.085 |

Table 2. Summary of results of statistical tests. The interaction numbers refer to corresponding interactions described in Table 1 and depicted in Figure 2. Only values for variables with statistically significant results (P < 0.05) are reported. The chi-square values, degrees of freedom, and p-values of fixed effects were generated by likelihood-ratio tests of the full model with and without the explanatory variables. For a complete list of statistical tests performed see Tables S1 and S2. For visualization of specific effects see Figures S1 – S4. G7 and G10 refer to the gallers *Baccharopelma dracunculifoliae* and *Rachiptera limbata,* respectively. For example, the result in line one refers to interactions 14 and 25 shown in Table 1 and Figure 2: aphid frequency is negatively associated with ant frequency (link 14), but in treatment Live Gall Exclusion this negative relationship is weakened (link 25).

STAR Methods

**RESOURCE AVAILABILITY**

***Lead contact***

Further information and requests should be directed to and will be fulfilled by the lead contact, Milton Barbosa (miltonbsjunior@ufmg.br).

***Materials availability***

This study did not generate new unique reagents. More detailed information about plant and animal species are listed in this work and will be made available by the lead contact upon request.

***Data and code availability***

* The raw data supporting the results have been deposited at Dryad Digital Repository and is publicly available as of the date of publication. DOI is listed in the key resources table.
* This paper does not report original code. Model structures are available below in the Quantification and Statistical Analysis section.
* Any additional information required to reanalyse the data reported in this paper is available from the lead contact upon request.

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

***Study Site***

This study was conducted at Serra do Cipó, in southeast Brazil, in the Cerrado biome. This region is characterised by quartzitic soils covered by rocky grasslands, with a predominance of herbs and shrubs 29. It has a Cwb Köppen climate type, with dry winters and rainy summers 29. The average annual rainfall is between 1250 and 1550 mm, and the average temperature ranges from 15.1 to 20.7ºC 29. 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.

***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 29. *B. dracunculifolia* has a key role in natural succession and regeneration and is, therefore, important in terms of biodiversity and ecosystem functioning 29. 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, *Baccharopelma dracunculifoliae* (Sternorrhyncha: Psyllidae)is the most common 16,30. 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. The galls remain attached to the plant after dehiscence and gradually become dry and woody. Both hatched and non-hatched galls of *B. dracunculifoliae* are occupied by many inquiline invertebrates, such as ants, spiders, aphids, etc. 17. These hatched galls can trigger indirect effects that feedback to the galler modifying its interactions with other species 15. At least ten parasitoid species have been reared from galls of *B. dracunculifoliae* 16 and parasitism rates are around 45% 17. When the parasitoids emerge, they leave a characteristic exit hole on the gall wall 31, 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 18 (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 18,19 (MB *pers. obs*). It has been found that ants reduce the number of *B. dracunculifoliae* nymphs per gall, and aphids reduce *B. dracunculifoliae* gall size because aphids compete with the galler for sap assimilates and young leaves in terminal buds 18. In addition, the presence of ants and aphids on *B. dracunculifolia* decreased the abundance of other free-feeding herbivores, and the presence of aphids decreased plant shoot growth 19. Ants tending aphids can have a direct negative impact on herbivores 32. 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 33,34.

**METHOD DETAILS**

***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:

(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 removedby direct collection. When setting up the experiment, we excluded a total of 1,861 live galls of the galler *B. dracunculifoliae* from the 16 individuals (Average = 116.312). Galls were excluded from all plant individuals and most had a similar number of galls. 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 (433 galls in total; Average = 27.062). Weekly observations were performed to exclude newly hatched galls;

(iv) *Control* – To emulate plant response to mechanical damage, non-galled leaves were removed in an equal amount to the average number of galls collected in treatments (ii) Live Gall Exclusion and (iii) Hatched Gall Exclusion.

***Monitoring and data collection***

During the study period (August - September 2015), we 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*– we performed weekly observations on the plant individuals during the study period, starting a week after setting up the experiments. 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. We quantified the number of individuals of each species (or morphospecies) of predators (e.g., spiders, lady-birds, praying mantids) and free-feeding insect herbivores, as well as the number of branches occupied by ants (ant frequency), and terminal buds occupied by aphids (aphid frequency). 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, we quantified the abundances of *B. dracunculifoliae* galls, and hatched *B. dracunculifoliae* gall (except in the treatments in which they had been excluded). We 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*. We also quantified the abundance of an apical gall induced by *Rachiptera limbata* Bigot (Diptera: Tephritidae) on the same branches. We chose to include the galler *R. limbata* in the study because in a previous experiment (Barbosa et al. 2017), 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, we collected up to 15 live galls of each galler species, *B. dracunculifoliae* and *R. limbata* from the same three branches (up to five of each type per branch, depending on gall availability). They were stored individually and taken to the lab for dissection. To calculate parasitism rates, we dissected the galls and quantified the proportion of parasitised and unparasitised nymphs per gall through the presence of “mummies” (parasitised galler nymphs). We also determined the mortality rate of parasitised and unparasitised galler nymphs, and aphid inquilinism (aphids per gall; not to be confused with “aphid frequency” on branches) in the *B. dracunculifoliae* galls. Before dissection, galls were also measured for width and length to calculate their volumes - as an indicator of performance - according to their shapes (Volume = 4/3π [1/2 Length] [1/2Width]2, for ellipsoid galls), although parasitism is thought to increase gall size 17.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Besides the interactions directly detected and quantified through observation and gall dissection, we tested for density effects by comparing a node’s frequency, abundance or trait (gall volume and plant size) among the exclusion treatments (Figures 1 and S1-4, Table S1). To test for link modulation by the density of other groups we contrasted the detected pairwise links with the abundance or frequency of other groups, entering treatment as a covariate with interaction, and week, block and plant individual as random effects depending on the dataset used. This allowed testing for the effect of node removal on interactions (three-way interaction) as well as on interaction modifications (four-way interaction) by comparing treatments with regard to the slope of the relationship between an affected link and the affecting node – e.g., how aphid frequency (herbivory) varies with ant frequency in each treatment (Figures 1 and S1b, Table S2 – row 1, column 5). In some cases, treatment did not include all treatments – e.g., Ant Exclusion treatment is not included in the test of the relationship between aphid frequency and ant frequency among treatments (Table 2, Figure S1b).

We used generalised linear mixed effect models (GLMMs) with Poisson errors for count data and binomial errors for proportion data 35 or, when it was not possible to obtain a satisfactory model fit, we fitted linear mixed effect models (LMMs) after square-root or log-transforming the data when necessary to improve the homoscedasticity of residuals. The structures of the maximal models are shown below in the Model Structures section. We used the lme4 package in R 36 to fit the models. To visualise the relationship between variables, we used the R package Visreg and calculated confidence intervals with bootstrap via bootpredictlme4 (Figures S1-4). We checked the GLMMs for over-dispersion of residuals using the function overdisp.glmer (RVAideMemoire Package). 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 the ones with lower Akaike Information Criteria (AIC) score. We performed simplifications of the maximal models by removing non-significant fixed effects to obtain a minimum adequate model 35. 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. We used the testInteractions function (phia Package) to perform Wald chi-square test for *post hoc* comparisons between treatments and to perform the pairwise comparisons of adjusted slopes with respect to the response variable for contrasts of the factor treatment.

***Node modulation***

We used data from the weekly observations to compare treatments in terms of the species and abundance of predators and free-feeding insect herbivores (LMMs, Table S1), as well as the number of branches occupied by ants (ant frequency; GLMM), terminal buds occupied by aphids (aphid frequency; GLMM), and shoot growth (LMM). We entered treatment as a fixed effect, week as a covariate with interaction and block and plant individual as random effects. Using the dissection dataset, we tested the effect of the node exclusion treatments on the relationship between the volume of *B. dracunculifoliae* gall and nymphs per gall (LMM). We also tested the relationships between inquilinism (aphid/gall) and gall volume (LMMs) and inquilinism and nymph mortality of *B. dracunculifoliae* galls, as well as mortality of parasitized nymphs (GLMMS). We entered treatment as a covariate with interaction in all models. We used block and plant individual as random effects.

***Link modulation***

To test for link modulation by the density of other groups we used the observation dataset to contrast aphid frequency (aphid-plant interaction) with herbivore and predator abundance, and ant frequency, entering treatment as a covariate with interaction, and week, block and plant individual as random effects in the LMMs (Table S2). Also, for the analyses of the effect of node removal on interactions (three-way interaction) as well as on interaction modifications (four-way interaction) we looked for variation among treatments in the slope of the relationship between pairs of variables. With the dissection data we compared inquilinism (aphid/gall) between parasitized and unparasitised galls (LMM) and also parasitism rates of *B. dracunculifoliae* and *R. limbata* galls among treatments (GLMMs).Combining the observation plus dissection datasets, we fit LMMs for the response variables abundance of *B. dracunculifoliae* and *R. limbata* galls, and aphid inquilinism, against the explanatory variables (abundance of predators and free-feeding insect herbivores; ant frequency; aphid frequency; and abundance of hatched *B. dracunculifoliae* galls). To test the relationship between parasitism of gallers *B. dracunculifoliae* and *R. limbata* against those same explanatory variables, we were not able to fit GLMMs, and instead we used general linear models (GLMs) with binomial distribution. All models had treatment as a covariate with interaction. We only entered block as random effect since in merging the observation and dissection datasets, the observation data had to be combined across weeks.

***Model Structures***

Node modulation:

*A) Observation data:*

i) Model<- glmer (or lmer) (node ~ treatment : week + (1 | block/ individual))

Nodes:

Ants (frequency on branches) - glmer with Poisson errors

Aphid (frequency on branches) - glmer with Poisson errors

Herbivores (richness and abundance) - lmer

Predators (richness and abundance) - lmer

ii) Model<- lmer (Host plant (shoot growth) ~ treatment + (1 | block))

*B) Dissection data:*

i) Model<- glmer (or lmer) (node ~ inquilinism (aphid/gall) : treatment + (1 | block/individual))

Nodes:

Galler G7 (gall volume) - lmer

Galler G7 (nymph mortality) - glmer with binomial errors

Parasitoids of Galler G7 (mortality of parasitized nymphs) – glmer with binomial errors

ii) Model<- lmer (G7 gall volume ~ G7 nymph abundance : treatment + (1 | block/individual))

Link modulation:

*A) Observation data:*

i) Model<- lmer (affected link ~ affecting node : treatment + (1 | week) + (1 | block/individual))

Affected link:

Aphid-Host Plant (aphid frequency on branches)

*B) Dissection data:*

i) Model<- glmer (affected link ~ treatment + (1 | Block))

Affected links:

Parasitoid-Galler G7 (parasitism of the galler G7) - binomial errors

Parasitoid-Galler G10 (parasitism of the galler G10) - binomial errors

ii) Model<- lmer (aphid inquilinism ~ G7 parasitism (binary) : treatment + (1 | Block/individual))

*C) Observation + Dissection data:*

i) Model<- lmer (affected link ~ affecting node : treatment + (1 | Block))

Affected links:

Aphid-Galler G7 (aphid inquilinism)

Galler G7-Host plant (gall abundance)

Galler G10-Host plant (gall abundance)

ii) Model<- glm (affected link ~ affecting node : treatment)

Affected links:

Parasitoid-Galler G7 (parasitism of the galler G7) - binomial errors

Parasitoid-Galler G10 (parasitism of the galler G10) – binomial errors

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