**Interaction Engineering: non-trophic effects modify interactions in an insect galler community**

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**Abstract**

1. Theory suggests that non-trophic interactions can be a major mechanism behind community stability and persistence, but community-level empirical data are scarce, particularly for effects on species interactions mediated through changes in the physical environment.
2. Here we explored how ecosystem engineering effects can feed back to the engineer, not only modulating the engineer’s population density (node modulation) but also affecting it’s interactions with other species (link modulation).
3. Gall induction can be viewed as ecosystem engineering since galls serve as habitat for other species. In a community-level field experiment, we generated treatments with reduced or elevated ecosystem engineering by removing or adding post-emergence galls to different plots of their host plant in the Brazilian Cerrado. We tested the effect of post-emergence galls on the galler, as well as on the galler-parasitoid and galler-aphid interactions.
4. The manipulation of post-emergence galls had little effect on the galler - abundance and survivorship were not affected, and gall volume changed only slightly - but modified interactions involving the galler, parasitoid wasps, and inquiline aphids. Aphid inquilines negatively affected density-dependent parasitism rates (interaction modification) likely by killing parasitised galling larvae. Post-emergence galls interfered with aphid inquilinism - likely by the provision of alternative habitat for aphids – and thus interfered with the negative effect of aphids on parasitism (modification of an interaction modification).
5. This work is one of the few studies to demonstrate experimentally the role played by environmentally-mediated interaction modification at a community level in the field. Moreover, by manipulating a species’ ecosystem engineering effect (post-emergence galls) instead of the species itself, we demonstrate the novel result that populations can be regulated by non-trophic effects initiated by their own activities that alter their interaction with other species. This reveals that indirect interactions mediated via the environment offer new pathways of feedback loops for population regulation. Our results indicate that interaction modification has the potential to be a key regulatory mechanism underlying interaction variation in nature, and play a major role in community structure, dynamics, and stability.

**Key words:** Aphids, Cerrado, ecosystem engineering, galling insects, indirect interactions, interaction modification, parasitoid wasps, parasitism

**Introduction**

Historically, the main focus of community ecologists has been on direct and negative interactions, such as competition and predation (Menge & Sutherland 1976; Connell 1983). However, indirect interactions can also play a major role in community structuring (Morris et al. 2004; van Veen et al. 2006). Indirect trophic interactions, which require the presence of an intermediate third species and are propagated through feeding relationships, are termed interaction chains or density-mediated indirect effects (e.g. trophic cascades, keystone species, apparent competition), and have been widely demonstrated (Wootton 1994, 2002; Morris et al. 2004; van Veen et al. 2006). Numerous shared physical conditions and resources can also mediate interactions between species in non-trophic ways (Wootton 1994), yet indirect interactions that are mediated non-trophically (e.g. via habitat, trait, or behaviour modification) remain largely overlooked (Ogushi 2008). Indirect non-trophic interactions can receive many different names when the effect takes place on species (nodes - e.g., facilitation, trait-mediated, ecosystem engineering, interference competition), and are called interaction modification when they affect the relationship (link) between two species (Wootton 1994, 2002). It has been demonstrated empirically and theoretically that indirect non-trophic interactions, such as the manipulation of plant chemistry by herbivores, and predators eliciting fear in their prey, can play a role in community structuring and ecosystem functioning (Kéfi et al. 2012). As a result, neglecting non-trophic interactions 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 non-trophic interactions is ecosystem engineering (Kéfi et al. 2012; Sanders et al. 2014; Coggan et al. 2018), a process whereby a species, by causing a physical change to the environment (biotic or abiotic), modulates resource availability for 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 the 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 alter engineer density and activity (Holt & Barfield 2013; Sanders et al. 2014).

Many studies have investigated non-trophic interactions (Jones et al. 1994, 1997; Odling-Smee et al. 2003; Bruno et al. 2003; Ings et al. 2009) but relatively few empirical studies have investigated their effects at a community level in the field (including Prasad & Snyder 2010; Kéfi et al. 2012, 2015; Hammill et al. 2015; Prugh & Brashares 2012; Wetzel 2016). Of these, very few have focused on non-trophic effects on changes in species interactions (interaction modification; Hammill et al. 2015; Prasad & Snyder 2010) rather than on changes in species density (e.g., interference competition, facilitation; Prugh & Brashares 2012; Wetzel 2016). Species can modify interactions via a trait change on one of the interacting species (predator causing fear on herbivore; plant-mediated interactions, etc; Hammill et al. 2015; Prasad & Snyder 2010) or via a change in the context of the physical environment (biotic and abiotic) where a pair of species interact. In the literature on interaction modification, empirical demonstrations of environment-mediated interaction modification (Wootton, 1993) are very scarce and mostly restricted to herbivores modifying habitat structure with consequences for refuge availability, and as a result mediating predation risk for other herbivores (e.g. Pagè et al. 2012). Research on ecosystem engineering has mostly focused on effects of habitat creation on nodes (mainly species density; Prugh & Brashares 2012; Wetzel 2016) rather than effects on interactions (environment-mediated interaction modification; Pagè et al. 2012). To the best of our knowledge, no previous work has looked at feedbacks of engineer effects via environment-mediated interaction modifications. The self modulation of interactions via non-trophic effects is relevant for population regulation and community stability. If it is possible for a species to modify its own interactions non-trophically, it reveals new possible pathways of feedback loops for population regulation.

Here we explored how ecosystem engineering effects can feed back 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 field system comprising a galler (an organism, in this case an insect, which induces an abnormal growth, or gall, on the plant, and develops inside it; Shorthouse & Rohfritsch 1992), the parasitoid wasps attacking the galler at the initial stages of gall development, and an aphid species that lives on terminal buds but also occupies the galls. The system is based on the host plant *Baccharis dracunculifolia* D.C. (Asteraceae), located in the Brazilian Cerrado. In our experiment we focused on 18 species directly linked to the focal galler species, including one aphid, 16 parasitoid species and the galler itself. More broadly the community of arthropods based on the host plant includes 15 ant species, nine predator species (mostly spiders and ladybirds), 41 free-feeding herbivorous insect species, and 17 galling insect species (M Barbosa, *unpublished data*). All of these insect and spider species interacting directly or indirectly with the host plant were able to contribute to the effects demonstrated here. The focal galler, *Baccharopelma dracunculifoliae* (Sternorrhyncha: Psyllidae), is extremely abundant and gall induction can be viewed as allogenic ecosystem engineering (action of the engineer on other living or non-living structure; Jones et al. 2010; 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 pre and post-emergence galls of *Baccharopelma 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; it colonizes fully developed galls, use them as shelter, and feeds on the plant tissues inside the gall wall. These inquiline aphids can lead to the death of galler larvae via an as yet unknown mechanism and there is evidence for preferential occupation of parasitised galls by aphids due to changes in gall shape that facilitate aphid access (Collevatti & Sperber 1997; Barbosa et al., *unpublished*).

We hypothesised that post-emergence galls on their host plants would mediate non-trophic interactions, interfering with parasitism of gallers by wasps and inquilinism of the galls by aphids, and thus feeding back to impact populations of the galler itself. There are numerous mechanisms through which galls could affect parasitism. For example, newly hatched gallscould interfere with parasitoid foraging behaviour increasing search times (see de Rijk et al. 2013, 2016). Alternatively, post-emergence galls could serve as a cue for parasitoids to identify suitable hosts from a distance. Parasitoids can exploit cues that originate from non-target instars of the host insect or from their host plants to increase their efficiency during host finding (Salerno et al. 2013). In terms of affecting inquilism by aphids, post-emergence galls could have a positive effect on the galling insect by increasing habitat availability for aphids and therefore lowering the pressure of inquilinism on pre-emergence galls. This would be the case if aphids showed a behavioural response where a fixed number of inquilines divide themselves among a number of occupied and unoccupied galls. However, in the longer term larger inquiline populations could build up where their resources are abundant, generating the reverse effect. Hence, there are diverse possible responses to post-emergence galls on their host plants through non-trophic interactions.

In a replicated field experiment, we excluded post-emergence galls from natural patches of the host plant and added them to other patches to test the effect of post-emergence galls on the galler, as well as on its interactions with parasitoid wasps and inquiline aphids. Specifically, we tested:

1. whether post-emergence galls interfere with the abundance, survivorship, and volume (a measure of performance) of pre-emergence galls as well as with parasitoid species richness and composition (node modulation);

(ii) whether post-emergence galls affect the strength of the galler-parasitoid and galler-aphid interactions (link modulation). We expected changes in the relationship between each pair following manipulation of post-emergence galls.

Since the post-emergence gall removal treatment could affect the nodes and links in multiple and potentially opposing ways depending on the mechanism (see above), directional predictions were not possible.

**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 15.1 to 20.7º 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 *Baccharis 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 *Baccharis dracunculifolia* frequently compose well-defined patches ranging from 18 to 12,000 m2 (Collevatti & Sperber 1997). Among the 17 species of gall-inducing insects recorded on *Baccharis dracunculifolia* in different locations, *Baccharopelma dracunculifoliae* is the most common (Fernandes et al. 1996) corresponding to approximately 83% of the galls (Barbosa et al. 2017). 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 *Baccharis 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 spatial blocks with three treatments each: exclusion and addition of post-emergence galls, and control. In the plots assigned to the exclusion treatment all post-emergence galls of *Baccharopelma dracunculifoliae* were removed through direct collection. Observations were made every two weeks to exclude newly hatched galls. The post-emergence galls are easily identified by the longitudinal fissure formed by the separation of the leaf edges, which are joined in pre-emergence galls. In the exclusion treatment, we removed an average of 24.13 (SD = 8.57) post-emergence galls per plant at the beginning of the experiment in July, 32.46 (SD = 8.69) in August, and 29.79 (SD = 9.72) in September. Post-emergence galls corresponded in average to 49.65% of all galls (pre-emergence + post-emergence) found on each plant at the beginning of the experiment. The post-emergence 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 post-emergence 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 in the lab with the aid of a slender brush and discarded. The other 10 plots served as controls. To emulate plant responses to mechanical damage, non-galled leaves were removed from addition and control treatments in an equal number to that of post-emergence 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 experiment was carried out from July – October 2013. The treatments were applied in July and gall abundance was determined twice in each plot, in August and October. We counted all full-sized or close to full-sized galls of *Baccharopelma dracunculifoliae* found in five half-metre branches haphazardly chosen around the crown of each of five individuals of *Baccharis dracunculifolia* within each plot. The individuals sampled in August were marked and were not re-sampled in October. In both sampling events, all fully developed pre-emergence galls found on the chosen branches were collected in each plot. The pre-emergence galls were reared in the laboratory under ambient conditions 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 70% 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. We only reared a single individual of an aphid parasitoid, *Aphidius colemani*Viereck (Hymenoptera: Braconidae, Aphidiinae), which we excluded from the analyses in order to avoid overestimating galler parasitism rate.

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 = 4/3π [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 pre-emergence 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 pre-emergence gall abundance and parasitoid richness across treatments (hypothesis i: node modulation) 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 included treatment as a fixed effect and month (August or October when the insects were sampled) as a covariate. We included block as a random effect in both models to allow for random intercepts for each block, reducing variation due to different conditions experienced by plants in different sites (blocks). 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. We fitted a general linear models (LM) for the response variables galler survivorship with pre-emergence gall abundance in a two-way interaction with treatment, and included month as a covariate without interaction. For gall volume, the linear model included only treatment and month as explanatory variables. Minimum adequate models and P-values were obtained, and models were checked through the same procedures described above for GLMMs. We also used the “adonis” function of the “vegan” package (Oksanen et al. 2018) to perform a permutational multivariate analysis of variance (PERMANOVA; Anderson 2017) to test the significance of the effects of each treatment on parasitoid species composition.

For the analyses of effects on relationships between species (hypotheses ii: link modulation) we tested for significant variation among treatments in the slope of the relationship between pairs of groups – e.g., how parasitism varied with pre-emergence gall abundance in each treatment. To test whether the interaction galler-parasitoid (pairwise interaction) was affected by post-emergence galls or aphid inquilinism (interaction modification) and whether these interaction modifications interfered with each other (modification of an interaction modification), we fitted a generalized linear model (GLM) with Binomial errors (Crawley 2013) including the response variable parasitism, with treatment, pre-emergence gall abundance, and aphid inquilinism, in a three-way interaction, and month as a covariate without interaction as the maximal model, since month was not the variable of interest and no differences among the three months were expected as a result of similar precipitation and the lack of seasonality of the galls. To visualize the relationship between the response and explanatory variables (Figure 1) we used the R package Visreg. We fitted a general linear models (LM) for the response variable inquilinism with either pre-emergence gall abundance in a two-way interaction with treatment, and included month as a covariate without interaction. Except for parasitism, all dependent variables and gall abundance were log-transformed to improve the homoscedasticity of residuals. We used Cohen’s *d* to calculate effect sizes for the treatments.

**Results**

Over the study period, we collected a total of 6,536 pre-emergence galls induced by *Baccharopelma dracunculifoliae*. The average abundance of pre-emergence 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 2,142 individual parasitoid wasps, representing 16 morphospecies (exclusion = 7; addition = 8; control = 10) within five families (Table S1 Appendix). We only quantified aphids within galls (inquilinism), so cannot quantify aphid abundance on plants.

*Effects on species (hypothesis i:* *node modulation)*

There was no statistically significant difference in abundance of pre-emergence galls, parasitoid richness (Table 1; Figs. S1 and S2 Appendix), or parasitoid species composition among treatments (PERMANOVA; F = 1,909; R2 = 0.041; p = 0.063). Pre-emergence gall abundance, but not parasitoid richness, was significantly higher in August than October (Table 1; Figs. 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; Fig. S3 Appendix). In October, gall volume was slightly greater in control (M = 218.5, SD = 108.5) than exclusion (M = 197.1, SD = 108; Table 1; t= 3.587, df = 2, p = 0.004; Cohen’s *d* = 0.19) and addition (M = 193.3, SD = 106.7; Table 1; t = 4.151, df = 2, p<0.001; Cohen’s *d* = 0.23) treatments.

*Effects on interactions (hypotheses ii:* *link modulation)*

Galler-parasitoid interaction: parasitism was lower in October than in August and was significantly affected by a three-way interaction among the variables treatment, pre-emergence gall abundance, and inquilinism (Table 1, Fig. 1). This three-way interaction indicates that the relationship between parasitism and pre-emergence gall abundance was negatively affected by aphid inquilinism (Fig. 1a). That is, aphid inquilines negatively affect density-dependent parasitism rates (interaction modification) - increasing pre-emergence gall abundance reduces parasitism more intensely if galls are more frequently occupied by inquiline aphids. However, this interaction modification by aphids was also weakened in the post-emergence gall exclusion and addition treatments (Fig. 1b, c), which means that post-emergence galls interfere in a non-linear way with the negative effect of aphids on parasitism (modification of an interaction modification; Fig. 3). The mean proportions of parasitised galls were 0.300 in the control (SD = 0.190), 0.215 in the exclusion treatment (SD = 0.151), and 0.231 in the addition treatment (SD = 0.169), therefore there was a mean reduction of 28% in parasitism on galls of plants with removed post-emergence galls and of 23% in plants with added post-emergence galls compared to the control

Galler-aphid interaction: aphid inquilinism was negatively correlated with pre-emergence gall abundance but with slight differences in slope among treatments (Table 1; Fig. 2). 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; Fig. 2). The mean number of aphids per galls was 1.408 in the control (SD = 1.785), 1.574 in the exclusion treatment (SD = 2.714), and 1.144 in the addition treatment (SD = 1.541). Overall, there was a 12% increase in inquilinism on galls of plants without post-emergence galls and a 19% decrease in plants with added post-emergence galls compared to the control. Aphid inquilinism was also higher in October than in August (Table 1, Fig. S4 Appendix).

**Discussion**

In our study, ecosystem engineering - the modification of the biotic physical environment - prompted environment-mediated interaction modification affecting the engineer’s interaction with other species (Fig. 3). By manipulating a species’ ecosystem engineering effect (post-emergence galls) instead of the species itself, we show how populations can be regulated by non-trophic effects initiated by their own activities. To the best of our knowledge, no previous work has looked at how an ecosystem engineer can change its own interactions with other species. Our study reveals overlooked pathways of feedback loops for population regulation that can drive population dynamics away from what should be expected from biotic effects only. These effects link species and the environment and show that species can impact each other via the environment (biotic and abiotic), increasing the potential for interactions within communities in general.

Our results indicate that engineer effects can perhaps be more prominent or important on interactions than on nodes/species density. The manipulation of post-emergence galls had little direct effect on the galler (node modulation) - galler abundance, and survivorship were not affected, and gall volume changed only slightly - but modified the galler-parasitoid-aphid interactions (link modulation). Looking at effects on nodes alone may not be enough to identify feedback loops because multiple co-occurring indirect effects can compensate or cancel each other out resulting in null net effects on populations. However, detecting interaction modification is more difficult than effects on nodes perhaps because it requires that all species are present together, and therefore studying them requires a more holistic approach (Wooton 2002). A great portion of studies on non-trophic interactions are confined to subsets of communities isolated in the field or in the lab. We carried out the experiments in the field, at a community level. All the species living on the shrubs were potentially involved with the indirect interaction and the resultant effects.

Ecosystem engineering in this experiment modified interactions non-linearly. Plants that had post-emergence galls either experimentally removed or added, experienced a reduced negative effect of aphid inquilinism on the interaction between the parasitoids and the galler (likely resulting from aphids killing parasitised galling larvae) – a modification of an interaction modification (Figs. 1 and 3). Also, the relationship between aphid inquilinism and pre-emergence gall abundance was weaker in plants with removed or added post-emergence galls (Fig. 2). The fact that both addition and exclusion treatments results in the same impact on the effect of aphid inquilinism on the parasitoid-galler interaction, and on the relationship between aphid inquilinism and pre-emergence gall abundance suggests a non-linear response of aphids to the abundance of post-emergence galls: both high (addition treatment) and low (exclusion treatment) abundance of post-emergence galls had the same effect on gall occupation by aphids. Besides being offset or reinforced by each other, non-trophic interactions may also vary due to density-dependent feedbacks (Jones et al 1997; Sanders & van Veen 2011). Non-trophic interactions could introduce non-linearity in the relationship between species if the magnitude of the effect is regulated by feedbacks that depend on the density of the species involved (Fig. S5). The non-linear density dependence of non-trophic interactions may be the key mechanism behind many reported changes in interaction sign and magnitude (Chamberlain et al. 2014). Our results suggest that interaction modification have the potential to be a key regulatory mechanism underlying community stability if interaction strengths change in response to environmental change precisely in order to buffer the effect on nodes.

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 post-emergence 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 post-emergence galls provided habitat for jumping spiders, which suppressed herbivorous arthropods. Here we move beyond a simple demonstration of non-trophic interaction effects through changes in density of other species to reveal the role of non-trophic effects in interaction modification, specifically through initiating feedbacks by affecting the engineer’s interactions with other species, and consequently playing a key role in community regulation. We show that non-trophic interactions triggered by post-emergence galls appear to change with abiotic and biotic conditions, such as the abundances of other species, or due to overlap with other non-trophic interactions. We discuss these effects and their implications for community stability in detail below. Although our experiments have documented the patterns, we can only predict from our detailed knowledge of the system, the mechanisms behind these patterns. This is because to investigate the patterns we would have had to carry out sampling that would have impacted on the patterns themselves. Furthermore, determining pathways of indirect effects is not a trivial task since some of the observed effects may be due to a combination of indirect effects, or they can involve effects propagating through several intermediate species.

*Interference among non-trophic interactions*

If indirect effects within a network change interactions and interfere with each other, 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; see Fig. S5 in Appendix for a putative diagram of possible outcomes of non-trophic effects on interactions). 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 & Soluk 2006). These results suggest that ecosystem engineering may have a much more profound influence on community structure than we realise, which will only be detected if we investigate interaction modifications.

In the present study, we identified some concurring effects that could potentially amplify or nullify each other. For example, there was a higher density of aphids inside galls when gall abundance was lower (Fig. 2). As a result, at lower gall abundances, saturation of galls occupied by aphids would affect parasitism more strongly. The negative effect of aphid inquilinism on parasitism (Fig. 1a) indeed suggests that aphids could lower parasitism by killing the parasitised larvae. In addition, there is evidence for preferential occupation of parasitised galls by aphids due to changes in gall shape that facilitate aphid access (M Barbosa, *unpublished data*). This reinforces the idea that post-emergence galls may have a significant positive impact on parasitism depending on whether post-emergence galls, by increasing habitat availability, reduced the colonisation of pre-emergence galls by inquilines. However, we were not able to assess the degree to which this happened, as collecting post-emergence galls would have interfered with the experimental treatments. In summary, as species densities vary, different combinations of overlapping effects will modulate interactions, leading to non-linear dynamics.

*Potential impacts on community structure and stability*

The experimental manipulation of post-emergence galls influenced the links, changing the interaction magnitude, whilst population densities (nodes) were unaffected. These results suggest that due to their flexible nature, non-trophic interactions could play a key role in stabilising our system, as has been suggested in other food webs (see Van veen et al. 2005; Hammill et al. 2015). Our results illustrate the numerous ways by which non-trophic interactions can mediate changes in the food web, 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; Barbosa et al. 2017). In our interaction network (Fig. 2) for instance, interaction modification by post-emergence galls is dependent on the galler population density. Whether the effect is positive or negative on the interactions, it depends on post-emergence gall abundance, which ultimately depends on pre-emergence gall abundance. Thus, the feedbacks are not of fixed direction but work as a switch that can increase or decrease the interaction strength according to population sizes.

The fact that a product of ecosystem engineering, such as a post-emergence gall, can play a role in interaction regulation suggests that such indirect effects may be far more widely spread and influential than is realised. Every species has the potential to contribute to non-trophic interactions, whether via ecosystem engineering or other means (e.g., facilitation, interference competition, trait and behaviour modification). It is therefore very likely that a complex network of non-trophic interactions is constantly modifying trophic 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), primarily because of the immense complexity of fully documenting non-trophic interactions within a community. Our results provide the first empirical evidence to show that such an approach is both feasible and essential.

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**Author contributions statement:** 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.

**Data accessibility statement:** We confirm that, should the manuscript be accepted, the data supporting the results will be archived in an appropriate public repository and the full data citation will be given in the reference list.

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**Table**

|  |  |  |
| --- | --- | --- |
| 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: χ2(2)= 0.560, p = 0.756 |
|  | Treatment | glmm: χ2(2)= 2.589, p = 0.274 |
|  | Month | glmm: χ2(1) = 80.652, p < **0.001** |
|  |  |  |
| Parasitoid richness | Treatment : Month | glmm: χ2(2)= 0.045, p = 0.873 |
|  | Treatment | glmm: χ2(2) = 0.792, p = 0.063 |
|  | Month | glmm: χ2(1)= 0.620, p = 0.431 |
|  |  |  |
| Parasitism | Treatment : Gall abundance : Inquilinism | glm: χ2(2) = 410.44, p < **0.001** |
|  | Month | glm: χ2(1) = 434.59, p < **0.001** |
|  |  |  |
| Galler survivorship | Gall abundance : Treatment | lm: F2,9 = 1.125, p = 0.333 |
|  | Treatment | lm: F2, 9 = 0.177, p = 0.837 |
|  | Gall abundance | lm: R2(adj)= 0.568 , F1,9 = 4.215, p = **0.045** |
|  | Month | lm: R2(adj)= 0.540, F1,9 = 10.572, p = **0.002** |
|  |  |  |
| Gall volume | Treatment : Month | lm: F2,9 = 3.259, p < **0.001** |
|  |  |  |
| Inquilinism | Gall abundance : Treatment | lm: adjusted R2= 0.444 , F2,9 = 3.378, p = **0.047** |
|  | Month | lm: adjusted R2= 0.231, F1,9 = 4.895, p = **0.034** |

**Figure legends**

Figure 1. Relationship between parasitism and abundance of pre-emergence galls induced by *Baccharopelma dracunculifolia,* according to distinct levels of aphid inquilinism (aphids / pre-emergence gall) in treatments (a) control, (b) exclusion of post-emergence gall, and (c) addition of post-emergence gall. Solid lines represent fitted GLM models

Figure 2. Relationship between density of inquiline aphid and abundance of pre-emergence galls of *Baccharopelma dracunculifolia* in distinct treatments. Solid lines represent fitted Linear Models

Figure 3. Interaction network based on field experiments on the host plant *Baccharis dracunculifolia* showing positive (+) and negative (-) effects categorised into three main types: node modulation (black arrows), which involves pairwise trophic (solid arrows) and non-trophic interactions (dashed arrows); and link modulation, which involves three-way interactions (interaction modification; dashed red arrows) or four-way interaction (modification of an interaction modification; dashed blue arrow). The numbers indicate (1) parasitism, (2) aphid inquilinism, (3) death of parasitized larvae, (4 and 5) unknown interaction mechanism – likely increased habitat provision for aphids

**Figures**

Figure 1. Relationship between parasitism and abundance of pre-emergence galls induced by *Baccharopelma dracunculifolia,* according to distinct levels of aphid inquilinism (aphids / pre-emergence gall) in: (a) control, (b) exclusion of post-emergence gall, and (c) addition of post-emergence gall, treatments. Solid lines represent fitted GLM models. In (a) aphid inquilines negatively affect density-dependent parasitism rates (interaction modification) - increasing pre-emergence gall abundance reduces parasitism more intensely if galls are more frequently occupied by inquiline aphids. However, this interaction modification by aphids was weakened in (b) and (c), the post-emergence gall exclusion and addition treatments, which means that post-emergence galls interfere in a non-linear way with the negative effect of aphids on parasitism.

a) Control



b) Exclusion of post-emergence galls



c) Addition of post-emergence galls



Figure 2. Relationship between aphid inquilinism (aphids / pre-emergence gall) and abundance of pre-emergence galls of *Baccharopelma dracunculifolia* in distinct treatments. Solid lines represent fitted Linear Models



Figure 3. Interaction network based on field experiments on the host plant *Baccharis dracunculifolia* showing positive (+) and negative (-) effects categorised into three main types: node modulation (black arrows), which involves pairwise trophic (solid arrows) and non-trophic interactions (dashed arrows); and link modulation, which involves three-way interactions (interaction modification; dashed red arrows) or four-way interaction (modification of an interaction modification; dashed blue arrow). The numbers indicate (1) parasitism, (2) aphid inquilinism, (3) death of parasitized larvae, (4 and 5) unknown interaction mechanism – likely increased habitat provision for aphids.

