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RESEARCH ARTICLE

Which traits optimize plant benefits? Meta-analysis on the effect of partner traits on the outcome of an ant–plant protective mutualism

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Abstract

1. Theoretical models on mutualism dynamics predict that partner traits may influence the outcome of mutualistic interactions. However, most empirical data on this issue is restricted to case studies, limiting our ability to reach a more widespread comprehension of the role of partner traits on the dynamic of mutualisms.
2. We investigated how the outcome of protective mutualisms between ants and plants bearing extrafloral nectaries (EFNs) is influenced by the traits of EFNs and ants feeding on EFNs. We used a meta-analytical approach based on 35 studies investigating the effect of ant attendance on the herbivores and reproductive performance of EFN-bearing plants. We evaluated how variation in the EFN vascularization and location on plants and the ant aggressiveness can modulate the effect of ant attendance on the plants.
3. Both plant and ant traits investigated here drove the outcome of the protective mutualism for EFN-bearing plants. Plants exclusively bearing EFNs near reproductive organs benefited more from ant attendance than plants bearing EFNs on vegetative or vegetative and reproductive organs. Ants had a higher positive impact on the reproductive performance of plants bearing non-vascularized EFNs than plants bearing vascularized EFNs, although their effects on herbivores had been similar in both plant types. Regarding the ant behaviour, plants often attended by more aggressive ant species had a higher reproductive performance than plants often attended by less aggressive ones.
4. *Synthesis*. Our results highlight that the selective pressures and evolutionary routes in ant-plant protective mutualisms may depend on the pool of traits exhibited by partner species. Although some studies have already reported some impact of species traits on the outcome of ant-plant mutualisms, this is the first time that a generalization about the role of species traits on the net balance of ant attendance was proposed. Due to this generalization, it was possible to advance our knowledge about the evolution of facultative mutualisms by showing

that the role of species traits on the mutualistic outcome can vary in intricate ways due to a particular trait combination found among partners in communities where the interactions are embedded in.

KEYWORDS
ant behaviour, ant-plant interaction, ecological interaction, extrafloral nectaries, facultative mutualism, plant defences

1 | INTRODUCTION

The outcome of mutualistic interactions is markedly variable in space and time (Bronstein, 1994, 2015). Several studies investigated the role of biotic and abiotic factors on such variation (e.g. Kersch & Fonseca, 2005; Maron et al., 2014). However, there is still no consensus about how and to what extent each factor influences the outcome of different types of mutualistic interactions. Specifically, in generalized mutualisms (sensu Bronstein, 2015), it has been proposed that the identity and, consequently, the traits of partner species can be important factors driving the outcome of mutualistic interactions for each partner (Heath & Sithcombe, 2014; Palmer et al., 2010; Stanton, 2003). This happens because generalized mutualisms involve several phylogenetically non-related species on each side of the interaction that largely differ in the morphological, behavioural or physiological traits mediating mutualistic interactions (Stanton, 2003). Such trait variation allows individuals of interacting species to vary in their ability to explore and provide resources and services to their partners (Dunkley et al., 2020; Heath & Sithcombe, 2014; Stanton, 2003). There is a growing number of case studies evaluating the role of species traits on the outcome of many different types of mutualistic interactions, such as cleaning, protective and dispersal mutualisms (e.g. Dehling et al., 2014; Melati & Leal, 2018; Sazima et al., 2010). In addition to their important contributions to the understanding of the dynamics of each mutualistic interaction, they also show that the selective pressures affecting these types of mutualisms differ. This marked variation in the types of mutualisms in case studies prevents us from drawing generalizations about the influence of partner species traits on the mutualistic outcome, how those outcomes vary in response to changes in the pool of partner species and the role of such influence on the evolutionary trajectory of different mutualistic traits (but see Stanton, 2003). Protective mutualisms between ants and plants bearing extrafloral nectaries (EFNs hereafter) represent an ideal system to investigate general patterns involving the effect of partner traits on mutualistic outcomes. In this mutualistic system, plants secrete extrafloral nectar that attracts predator ants that repel or eat herbivores while foraging on the plants (Marazzi et al., 2013). Thus, ants benefit from the consumption of extrafloral nectar (Byk & Del-Claro, 2011; Calixto et al., 2021; Lach et al., 2009), while plants benefit from the protection offered by ants (Chamberlain & Holland, 2009; Leal & Peixoto, 2017; Rosumek et al., 2009). Despite the similarity in how ants and EFN-bearing plants interact (i.e. ants are attracted to plants due to the nectar offered, while plants gain protection through herbivory predation by ants), plant traits involved in the provisioning of extrafloral nectar and the ant traits associated with the protective services against herbivores are highly variable among the species involved in this type of mutualism. This happens because this is a mutualism widely spread in nature, involving hundreds of ant and plant species occurring in many habitats (Weber & Keller, 2013). Such taxonomic diversity translates into an impressive variation in the anatomical structure, developmental origin and patterns of nectar secretion in EFNs across plant species worldwide (see Diaz-Castelazo et al., 2005; Machado et al., 2008; Melo, Córdula, et al., 2010; Nogueira et al., 2012, 2013). Regarding the ants, EFNs can be accessed by any ant species capable of foraging in the vegetation, resulting in a great variety of ant species visiting EFN-bearing plants (Barton, 1986; Bentley, 1976; Keeler, 1979). Therefore, like the plants, ants involved in this protective mutualism largely differ in their morphological and behavioural traits and such differences determine how they interact with herbivores and plants.

Among the traits mediating the interaction between ants and EFN-bearing plants, three features may be particularly important drivers of the plant net benefit: EFN spatial distribution within the plant, EFN vascularization and ant aggressiveness. The spatial distribution of EFNs on plants can determine the spatial distribution of the visiting ants on the plant surface. The presence of EFNs only on vegetative organs improves the ant patrolling on leaves and stems, which in turn, can benefit the plants by repelling herbivores on these organs (e.g. Fuentes & Marquis, 1999; Nascimento & Del-Claro, 2010) and/or distracting ants from flower resources (Villamil et al., 2019; Wagner & Kay, 2002). However, some plant species also possess EFNs adjacent to their reproductive organs (such as EFNs on the inflorescence peduncle or the outermost part of sepals), with some exhibiting EFNs exclusively in the reproductive regions (see Weber & Keller, 2013). Two opposite outcomes of ant attendance may occur for these species. First, the presence of EFNs adjacent to reproductive organs may negatively affect plant reproduction via ant interference with pollination (Ness, 2006; Villamil et al., 2018, 2019, but see Santos & Leal, 2019). In contrast, this potential negative effect can be absent or even offset by the ant protection of the reproductive organs against florivores and frugivores (e.g. Del-Claro et al., 2013; Ruhren, 2003; Wagner & Kay, 2002) and/or by increasing cross-pollination success (e.g. Altshuler, 1999; Sousa-Lopes et al., 2020). Since there is empirical evidence supporting both possibilities, a general evaluation compiling such evidence is needed to understand whether a consensus about the overall effect of EFN location on the benefits received by plants through ant attendance can be reached.

In addition to the location of EFNs, vascularization is another EFN trait that can mediate the benefits of ant attendance to plants. Extrafloral nectaries can range from highly vascularized structures to EFNs lacking vascularization (Diaz-Castelazo et al., 2005; Melo, Córdula, et al., 2010; Melo, Machado, et al., 2010; Nogueira et al., 2013). Due to the connection with the plant hydraulic system, vascularized EFNs are expected to secrete a higher volume or high-quality nectar than non-vascularized ones (Ávila-Agaéz et al., 2018; Delgado et al., 2011), especially when plants are under herbivore attack. Several studies have shown that extrafloral nectar traits (including volume and sugar and/or amino acids concentration) drive plant attractiveness to ants (e.g. Hernandez-Cumplido et al., 2016; Jones & Koptur, 2015; Kost & Heil, 2005; Nogueira et al., 2021), with plants secreting more valuable extrafloral nectar being more prone to benefit from ant attendance (e.g. Kost & Heil, 2005; Pacelhe et al., 2019). Therefore, plants bearing vascularized EFNs may be more attractive to ant bodyguards and benefit more from the interaction than plants with non-vascularized ones.

Regarding the effect of ant aggressiveness on the mutualism outcome of EFN-bearing plants, it is important to note that ant communities worldwide are strongly structured by dominance hierarchies based on behavioural differences in communication and aggressiveness between ant species (Cerdá et al., 2013; Savolainen & Vepsäläinen, 1988). In these hierarchies, overly aggressive species (referred to as dominant species from now on) exert a strong influence on less aggressive, subordinate species (Cerdá et al., 2013). Dominant species typically exhibit nest-raiding behaviours, are aggressive against other ants, use chemical defences and have caste-specific defensive roles (Cerdá et al., 2013). In contrast, subordinate species commonly forage solitarily and explore resources furtively (Cerdá et al., 2013). Due to their behaviour, dominant ant species are widely considered more efficient bodyguards in protective mutualisms with plants (Apple & Feener, 2001; Leal & Peixoto, 2017; Xu & Chen, 2010; but see Melati & Leal, 2018). Therefore, plants visited more frequently by dominant ant species can benefit more from ant attendance than those visited by subordinate species.

In the last two decades, some empirical compilations quantifying the effect of ant bodyguards in plants indicated that ants have a general positive protective effect on plants (Chamberlain & Holland, 2009; Rosumek et al., 2009; Trager et al., 2010). However, it is important to note that these compilations involved many different types of ant-plant interactions, such as ants and plants bearing EFNs, ants and plants with domatia (nesting sites offered by the plants), and ants present in plants hosting aphids secreting honeydew. This variation in the types of association included in such compilations hinders a clear identification of the factors that may affect the dynamics of these mutualistic interactions since they involved groups of species that are subjected to very distinct ecological and evolutionary processes. For instance, mutualisms between ants and EFN-bearing plants have a much greater variation in the identity of species involved in the interaction than mutualisms between ants and plants with domatia, in which each plant interact with a few specialized ant species (Rico-Gray & Oliveira, 2017). In the same way, interactions between ants and plants with aphids involve an herbivore (i.e. the aphids) that affect how selection should favour plant traits that may attract ants (see more in Nelson & Mooney, 2022). In addition, mixing different types of ant-plant mutualism in the same analysis reduces our ability to find insights into how trait variation among the interacting species in different communities may affect the net benefit of the interaction to the EFN-bearing plants and how it has shaped the evolution of extrafloral nectary traits These previous compilations also did not consider how behavioural variations in ants, an important component that determines how ants should explore the visited plants (e.g. Anjos et al., 2017; Melati & Leal, 2018) and consequently influence the results of mutualisms between EFN-bearing plants and ants. To fill such gaps, we used a meta-analytical approach to evaluate how traits of EFN-bearing plants and their respective ant bodyguards can determine the outcome of this protective mutualism for plants. We evaluated four hypotheses. The first two are competing hypotheses regarding the effect of EFN location on the ants' effect on the plants: plants bearing EFNs adjacent to reproductive organs (i) benefit less or (ii) benefit more from ant attendance than the ones with EFNs only on vegetative organs, depending on the ants' overall effect on the pollination of such plants. The other two hypotheses are related to the effect of EFN vascularization and ant behaviour on plant fitness: (iii) plants bearing vascularized EFNs receive higher benefits from ant attendance than plants bearing non-vascularized EFNs and (iv) plants more often visited by aggressive dominant ant species benefit more by the protective ant services than plants visited more often by subordinate ant species.

In the literature, studies have investigated the effect of ants on EFN-bearing plants by using observations of the ant effect on herbivores' occurrence and their activity on plants and the effects of ants on the reproductive performance of EFN-bearing plants (see the studies used in the meta-analysis of Chamberlain & Holland, 2009; Leal & Peixoto, 2017; Rosumek et al., 2009). Although a negative effect of ants on herbivore activity may translate into a subsequent increase in plant fitness, it is also possible that the negative effects of ants on herbivores are offset over the plant's lifespan, not positively impacting plant fitness. Therefore, quantifying the ant effects on herbivores may yield relatively weak evidence of the benefit EFN-bearing plants receive from ants. Because of this, we evaluated the hypotheses described above by using two sets of metrics related to the ant effects on this mutualism: metrics representing the ant effects on herbivores and their activity and metrics representing the ant effects on plant reproductive performance.

2 | MATERIALS AND METHODS

2.1 | Data collection

To perform our meta-analysis, we focused on studies providing empirical data about the ants' impact on herbivores and/or on the performance of EFN-bearing plants. A database compiling studies that fit these requirements was provided by Leal and Peixoto (2017) in a previous meta-analysis that evaluated the role of water availability in driving the effect of ant attendance on the performance of EFN-bearing plants. Although their objective was different from ours, their database was built following a replicable and detailed protocol suitable for searches focusing on studies investigating the role of ant attendance on EFN-bearing plants. Since Leal and Peixoto's (2017) database included studies published in English between 1970 and September 2015, we updated this database by searching for new studies published between October 2015 and December 2019. For that purpose, we searched for papers in Scopus (www.scopus.com) and ISI Web of Knowledge (www.isiknowledge.com – core collection) databases, using the following key terms: 'extrafloral nectar*', 'ant-plant mutualism', 'plant AND biotic defen*e'. These keywords and search sites were the same as used by Leal and Peixoto (2017). We found 25 new studies published in English for this new search, added to the 126 studies compiled by Leal and Peixoto (2017).

After building the database with the 151 studies, we screened the abstracts and methods to select the studies that fulfilled the following inclusion criteria: the studies must have (1) used plant species bearing natural EFNs as model organisms, discarding all the studies artificially manipulating EFN occurrence or extrafloral nectar availability to ants; (2) evaluated the role of ant attendance on plants by contrasting two groups of plants: one freely accessible by ant species (control group) and another group in which ants were naturally absent or experimentally excluded from plants; (3) used native plant species as a model to prevent confounding effects specific to invasive species (see Traveset & Richardson, 2006); and (4) reported the sample size, the mean values and the standard deviation or standard error for metrics related to herbivore performance or plant reproductive success in groups of plants in which ants were present or absent (see more information about these metrics below). After this initial screening, 38 studies remained in our database. However, we excluded from our dataset three studies that used as models two fern species: *Pteridium aquilinum* and *Polypodium plebeium* (Compton & Lawton, 1992; Jones & Paine, 2012; Koptur et al., 1998; see Table S2). Ferns differ markedly from angiosperms in their structure and reproductive mode, making any comparison between these two groups potentially misleading. Then, we obtained 132 effect sizes from 35 studies undertaken in 31 different locations and using 42 EFN-bearing plant species belonging to 20 families of angiosperms as model species. When data were reported in figures in the original studies, we estimated the values for each sample using the *WebPlotDigitalizer* software (Rohatgi, 2021).

We classified our effect sizes into two groups according to the type of ant effect metrics described in the original study: metrics describing the ant effects on herbivores and metrics describing the ant effects on plant reproductive performance. The ant effects on herbivores included any metric describing ants' influence on herbivores' occurrence and their activity on plants (e.g. herbivore abundance, herbivore removal by ants, rate of leaf area consumption, rate of florivory – *N* = 47 effect sizes). Meanwhile, the ant effects on plant reproductive performance included all the effect sizes from metrics describing ant influence on the plant's reproductive performance (e.g. number of floral buds, number of fruits, number of seeds – *N* = 43 effect sizes). We described all the metrics extracted from each study and their classification in Supplementary Material (Table S1). Effect sizes describing the vegetative performance (e.g. growth rate, number of leaves) represented few cases in our dataset (*N* = 14 effect sizes, coming from only four studies). Although they represent important information regarding plant fitness, this small sample size would prevent us from building models including interactive effects that properly evaluate the predictions of our hypothesis. For this reason, we excluded the effect sizes related to plant vegetative performance from our database.

We used two approaches to gather information on EFN location and vascularization of the plant species included in our dataset. First, we searched for the necessary information in the original article included in our dataset. When some information was unavailable, we performed a second literature search considering the plant or ant species for which we needed information (e.g. Diaz-Castelazo et al., 2005; Melo, Córdula, et al., 2010; Melo, Machado, et al., 2010; Nogueira et al., 2013). Regarding the EFN location, we recorded if the plant species had active extrafloral nectaries on the vegetative organs only, the reproductive organs only or both. We considered EFNs to occur adjacent to the reproductive organs whenever the EFNs were located on the outermost part of the sepals, bracts, inflorescence and infructescence peduncles, or fruits and were not directly associated with flower resources to pollinators. We gathered this information for all 42 plant species included in our database. Regarding morphology, we identified if the EFNs were vascularized or non-vascularized for all 42 plant species included in our dataset. We classified the position of EFNs according to the information presented by the authors in the papers from which we took the effect sizes. Additionally, we confirmed the position of EFNs using the dataset available in the world list of plants with extrafloral nectaries (http://www.extrafloralnectaries.org/) and taxonomic descriptions of the focal species available in the literature. In all cases, the position of EFNs on plants was checked in morphological descriptions studies of the focal species itself. This approach is essential as the topological variation of extrafloral nectaries on plants varies significantly between species, even between closely related species (e.g. *Bignonieae* species; Nogueira et al., 2012). In contrast, the classification of EFN vascularization was based on information available in publications containing anatomical descriptions of the EFNs performed either in the same focal species of each study or in broader clades in which the focal species is included. This approach is possible because, within the same plant lineage in which EFNs evolved, vascularization (being or not being vascularized) is conserved throughout the clade (e.g. *Bignonieae*, *Chamaecrista*, *Inga*, *Macaranga*, *Qualea*). Also, as the anatomical description of EFNs for some groups is scarce in the literature, in some cases, we used descriptions of external morphology as a proxy to classify the vascularization of EFNs. This strategy was applied when the EFNs on plants were of the trichome type. In these cases, nectar-secreting trichomes (scale-like or capitate) were classified as non-vascularized extrafloral nectaries (e.g. Bignonieae species). For the other types of EFNs on plants, we considered the anatomy studies of these secretory structures to classify the EFNs as vascularized or not.

To evaluate the role of ant traits on the outcome of the interaction between ants and EFN-bearing plants, we determined which ant species more often interacted with the EFN-bearing plants in each study selected in our meta-analysis. We considered as the main ant visitor the ant species interacting with each plant species at a frequency higher than 60% of the observations in each study (see Leal & Peixoto, 2017 for more details). To identify the main ant species visiting the EFN-bearing plants in each study, we selected the original papers in our database that reported the frequency at which different ant species were observed on the plants about the total number of observations. A total of 31 out of 35 studies remained (n = 116 effect sizes). We only considered studies in which the authors recorded the presence/absence of different ant species on plants, regardless of the number of workers of each ant species per plant. So, even though other ant species may contribute in some way to the outcome of ant-plant defensive mutualism for the plants, it is reasonable to expect that most of the effects reported in each study are a consequence of the defensive service provided by the ant species that were patrolling the plants in more than half of the observations. We applied this approach because it is known that the most frequent mutualistic species have the highest influence on their partners' performance, determining the central tendency of the estimates obtained for the outcomes of mutualism in empirical studies (Fagundes et al., 2017; Vazquéz et al., 2005). Additionally, although the studies investigating the patterns of EFN-bearing plant attendance by ants describe that a given plant species can be visited by several ant species, each individual within a population tends to be monopolized by a few ant species (Nogueira et al., 2015). Indeed, the main ant species interacting with the model plants in our dataset visited the plants at a frequency superior to 70%, on average. Most studies in our dataset did not provide behavioural information on the main ant species visiting the EFNs. Therefore, we used a generally accepted classification of ant aggressiveness based on the competitive hierarchical organization of ant communities worldwide for ant aggressiveness (Cerdá et al., 2003). This classification uses information about workers' morphology, colony size and foraging behaviour to classify ant species belonging to different genera worldwide as top-dominant, sub-dominant or subordinate species (Andersen, 1995; Cerdá et al., 2003; Lach et al., 2010). However, in communities where top-dominant ants are absent, sub-dominant species can play the same functional role as top-dominant species, including their potential role as high-quality bodyguards of EFN-bearing plants (Cerdá et al., 2013). For this reason, we grouped top-dominant and sub-dominant ants into a single 'dominant' group. According to this classification, we classified the main ant species visiting the plants in each study into two groups, dominant and subordinate, in which dominant species are more aggressive than subordinate ones. See Leal and Peixoto (2017) for more information regarding this classification. Considering all studies, we had 15 plant species most frequently visited by a main dominant ant species, 17 plant species most frequently visited by a main subordinate ant species, four in which the authors did not provide information about the plant interaction frequency by different ant species and four in which the most frequent ant species interacted with the plants in less than 60% of all observations.

2.2 | Effect sizes and meta-analytical models

In each selected paper, we recorded the mean, the standard error and the sample size values of any variable representing herbivores' or plants' responses to ant-included/ant-excluded treatments. In studies in which the ant attendance effect was recorded recurrently over time, we used the mean values of plant response over all the sampled periods (1 case).

We used the log of the ratio of means (ln(RM)) as the metric of the effect size of ant attendance on herbivores and plant reproductive performance. This metric represents a standardized ratio between the means of two groups and it is commonly used to quantify the proportional change from experimental manipulation (Hedges et al., 1999). To make our data more intuitive, we calculated the ln(RM) differently for each kind of response variable measured in the original studies included in our database. For effect sizes describing the ant effect on herbivores and their activity, we calculated the ln(RM) values using the mean of the group in which ants were excluded, divided by the mean of the groups in which ants were allowed on the plants (control plants). Therefore, higher ln(RM) values indicate that ants are more efficient in reducing herbivore occurrence or herbivore activity on plants. For metrics representing the ant effects on plant reproductive performance, we calculated the ln(RM) values using the mean of the group in which ants were allowed onto the plants (control plants) by the mean of the group in which ants were excluded. It followed that higher positive ln(RM) values indicate that ant attendance results in a higher benefit to plant performance. Therefore, regardless of the metric type used in the original study, positive and higher values of ln(RM) indicate a higher probability that EFN-bearing plants benefit from ant attendance.

To estimate the overall ant effect on herbivores and the reproductive performance of EFN-bearing plants, we first built a meta-analytical multilevel model including the type of ant effect metrics (i.e. on herbivores or the plant performance) as moderator (sensu O'Dea et al., 2021). To evaluate the effect of EFN location, we built a similar meta-analytical model, including the interaction term between the type of ant effect and the location of EFNs on the plants (i.e. vegetative, reproductive or both organs) as a moderator. Finally, to evaluate the influence of EFN vascularization on plant benefits, we built a third model, including the interaction term between the type of ant-effect metrics and the vascularization of the EFNs (vascularized and non-vascularized) as moderators. We evaluated the role of EFN location and vascularization in separate models for two reasons. First, the moderators 'EFN location' and 'EFN vascularization' were not independent of each other ($\chi^2 = 14.52$, $df = 2$, $p < 0.001$). Second, there was no variation in the vascularization of EFNs in plant species bearing EFNs in both vegetative and reproductive organs. All the last-mentioned plant species possess vascularized EFNs. Hence, to include an interaction term in our models, we would need to discard an entire level of one of our moderators. This decision would harm the fit of our models and the generality of the biological results coming from them.

We used ln(RM) as the response variable for all the models described above and weighted it by the inverse of the variance associated with the ln(RM). If plant species bearing EFNs on reproductive organs benefit less from ant attendance, we would expect the mean ln(RM) to be higher in plant species with EFNs located only on vegetative organs than in plants with EFNs on reproductive or both organs. Alternatively, if plant species with EFNs located on reproductive organs benefit more from ant attendance, we would expect ln(RM) to be similar or even lower in plants with EFNs located only on vegetative organs than in plant species with EFNs located on reproductive or both organs. If EFN vascularization improves the effect of ants on the plants, we would expect the ln(RM) value to be higher in plant species with vascularized EFNs than those with non-vascularized EFNs.

To evaluate the effect of ant behaviour on plant benefits, we also built a meta-analytical multilevel model, including the ln(RM) as the response variable and the interaction between the type of ant effect metrics and the aggressiveness behaviour of the main ant species visiting the plants (dominant or subordinate category) as moderator. If plants mainly attended by dominant ants benefit more from ant attendance, we would expect the ln(RM) value to be higher for plants mainly attended by dominant ant species than those attended by subordinate ant species.

We included four random factors in all models described above: study identity, habitat type, phylogenetic relatedness between plant species and plant identity. We included study identity as a random factor to control dependent effects among effect sizes extracted from the same study. Similarly, plant response to ant attendance can depend on environmental conditions (see Leal & Peixoto, 2017; Pringle, 2016). Therefore, we also included the habitat type as a proxy for the general environmental conditions faced by the plants in each study. We classified the habitat into four broad categories according to the habitat description provided by the authors in each study: grasslands ($n = 42$), shrublands ($n = 74$), tropical forests ($n = 10$) and temperate forests ($n = 21$).

Given that we estimated the effect of ant attendance on several plant species, we included the phylogenetic relatedness between plant species in our models because of the non-independence of species with common ancestry. For this purpose, we built an ultra-metric phylogeny comprising all 40 plant species using the V.PhyloMaker R package (Jin & Quian, 2019). We then transformed the ultra-metric tree into a variance–covariance matrix that reflects the phylogenetic relatedness among the plant species. The variance–covariance matrix was then inputted into our meta-analytical models (see below for a detailed description of each model) as a random variable. Finally, as we had more than one effect size extracted for the same plant species, we also included the plant identity as a random factor in the models.

We ran all the models using the *rma.mv* function in the metafor package in R (Viechtbauer, 2010). We ran the models excluding the intercept to obtain the estimated mean effect size for each moderator level in all cases. We also ran the models with the intercept to evaluate whether moderator levels differ from each other. We considered that effect sizes would differ statistically from zero when the 95% confidence intervals (CI) did not overlap zero.

2.3 | Publication bias and data heterogeneity

We used a modified version of Egger's regression (Nakagawa & Santos, 2012) to evaluate potential publication bias in our models. This test evaluates how independent meta-analytical residuals are from each other. If the Egger regression intercept differs from zero, this provides evidence of publication bias in the models.

We used the I^2 statistic to estimate effect size heterogeneity in our dataset (Nakagawa & Santos, 2012). I^2 allows us to partition the overall model's heterogeneity among different sources, indicating the percentage of variation explained by each random variable included in our models. Therefore, we estimated the I^2 values for study identity, habitat type, phylogenetic relatedness and plant species identity (Table 1). The sum of all I^2 values represents the total heterogeneity of the model (I^2_{total}) (Table 1).

TABLE 1 Summary of the Egger's test for each meta-analytical model

Moderator	Estimates	*t*-value	*p* value	Upper CI	Lower CI
None (overall ant effect)	−0.22	−2.23	0.03*	−0.42	−0.02
Herbivores × Plant performance	−0.20	−2.08	0.02*	−0.40	- 0.01
EFNs location	−0.22	−2.15	0.03*	−0.43	0.02
EFNs vascularization	−0.21	−2.11	0,04*	−0.41	−0.01
Ant behaviour	−0.20	−2.25	0.04*	−0.38	0.02

Abbreviation: CI, confidence interval.

* indicates the p values < 0.05.

3 | RESULTS

We obtained information about the EFN traits of 38 plant species belonging to 18 families. Fabaceae and Euphorbiaceae were the most common plant groups with 11 and 7 species, respectively. Regarding the distribution of EFNs on plants, 60.5% of the species have EFNs located only on vegetative organs (26 species, $N = 73$ effect sizes), 10.5% only on reproductive organs (4 species, $N = 19$ effect sizes) and 28.9% on both organs (12 species, $N = 40$ effect sizes). Most of the plant species in our database bear vascularized EFNs (78%). For the ant species visiting EFNs, the main ant visitor was distributed among 10 genera and four subfamilies (Formicinae, Myrmicinae, Dolichoderinae and Pseudomyrmicinae). *Camponotus* ants were the most frequent main visitors across studies (12 studies—34.3% of the effect sizes in which we obtained information on ant identity). Ants belonging to the *Monomorium*, *Tapinoma*, *Pseudomyrmex*, *Liometopum* and *Forelius* genera were the main visiting ants in only one study each. Dominant ant species were the main ant visitor in 78% of our studies, while subordinate ant species were the main attendant in 22%.

Pooling together all the metrics of ant effect on herbivores and plant performance, we found that ants visiting EFNs had an overall low positive effect on plants (estimated mean log(RM) values ± SE = 0.27 ± 0.08, 95% CI = 0.11 to 0.42). This overall effect was different from zero. The magnitude of ant effects on herbivores and plant performance differed markedly ($Q_{metric} = 121.60$, $df = 1$, $p < 0.001$—Figure 1a). On average, the magnitude of the ant effect on herbivores' occurrence and activity was 4.8 times higher than the ant effect on the plant's reproductive performance. Still, while the mean value of the ant effect on herbivores was greater than zero, the ant effect on the plant's reproductive performance was not different from zero (i.e. overlapped with zero; Figure 1a). Therefore, while the ant effect on herbivores is positive, indicating that ants commonly reduce herbivory on plants, it was negligible for plant performance, indicating that ants do not strongly improve plant reproductive output.

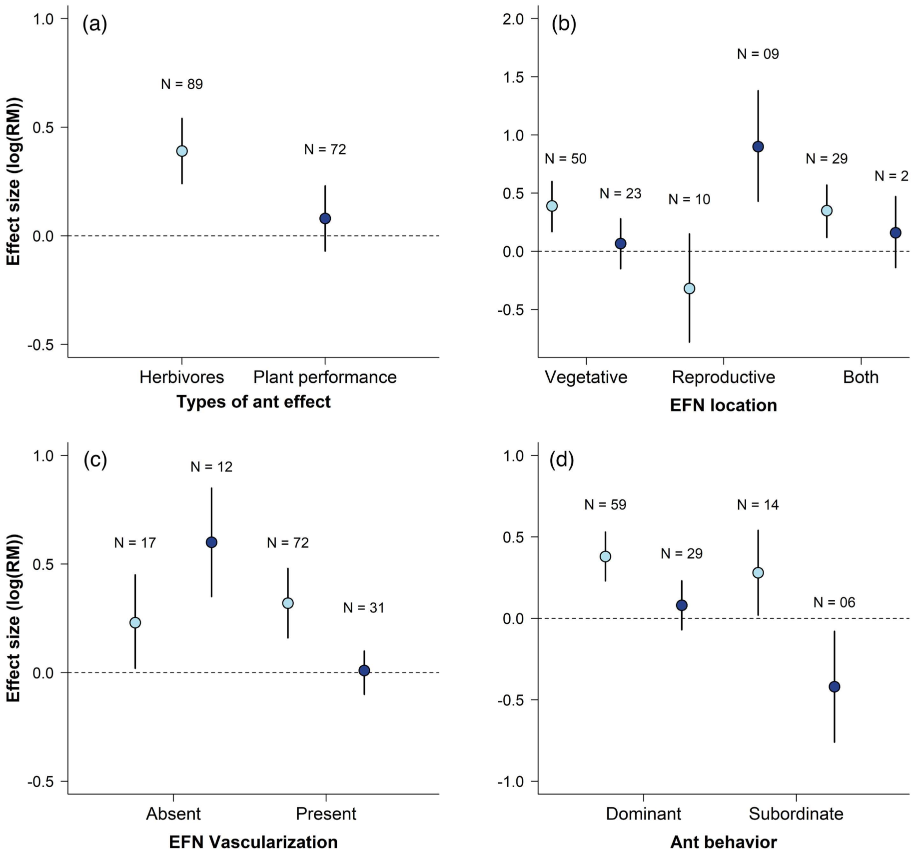

FIGURE 1 The effect of ant attendance on plants bearing extrafloral nectaries (EFNs) depends on the type of ant effect considered and on the traits of plant and ant species involved in the interaction. (a) represents the overall estimated mean effect of ant attendance to extrafloral nectaries (log(RM)) on the herbivores and the reproductive performance of plant species bearing extrafloral nectaries (EFNs). (b and c) represent the estimated mean effect of ants on the herbivores and reproductive performance of plants bearing EFNs on different organs (vegetative, reproductive organs or both) and vascularized and non-vascularized EFNs, respectively. Finally, (d) represents the mean estimated ant effect on the herbivores and the reproductive performance of plants mainly attended by dominant or subordinate ant species. Dots are the estimated mean effect size (log(Ratio of Means)), lines are the 95% confidence intervals, and the numbers above the bars are the number of effect sizes in each category. Light blue dots represent the estimated mean of ant effect on herbivores' performance and the dark blue dots, the mean estimated effect of ants on the plant reproductive performance. The dashed line is the reference value for the absence of ant effect (log(RM) = 0).

We detected an interaction between the location of EFNs on different plant organs and the magnitude of different types of ant effects on plants ($Q_{\text{metric*location}} = 216.85$, $df = 5$, $p < 0.001$). The magnitude of ant effects on herbivore activity was 1.2 and 1.1 times higher for the plants bearing EFNs on vegetative and both organs than for the ones with EFNs only in the reproductive organs (Figure 1b). Also, the effect of ant attendance on the activity of herbivores on the plants with EFNs only on the reproductive organs was not different from zero. Regarding the ant effect on the plant performance, it was 13 and 5.6 times higher for the plants bearing EFNs only on reproductive organs than for the plants bearing EFNs on vegetative and both organs, respectively (Figure 1b). Interestingly, the ant effect on the activity of herbivores on plants bearing EFNs on vegetative or both organs was not different from zero (Figure 1b).

We detected an interaction between the vascularization of EFNs and the magnitude of different types of ant effects on plants ($Q_{\text{metric*vascularization}} = 127.72$, $df = 3$, $p < 0.01$, Figure 1c). The magnitude of the ant effect on the herbivores was similar for plant species bearing vascularized and non-vascularized EFNs, with both values greater than zero (Figure 1c). However, the magnitude of the ant effect on plant performance in plant species with non-vascularized EFNs was 60 times higher than in plant species with vascularized EFNs (Figure 1c). For plants bearing vascularized EFNs, the estimated mean of the ant effect on plant performance did not differ from zero (Figure 1c). These results indicate that ants effectively reduced herbivore activity in plants bearing vascularized and non-vascularized EFNs but provided reproductive benefits only to plants with non-vascularized EFNs.

Regarding the effect of aggressiveness behaviour of the main ant visitor on plants, we observed an interaction between the ant behaviour and the type of the ant effect on plants ($Q_{\text{metric*behaviour}} = 131.92$, $df = 3$, $p < 0.001$, Figure 1d). The estimated magnitude of ant effects on the herbivores was positive for plants mainly attended by dominant and subordinate ant species and differed from zero in both cases (Figure 1d). Regarding the effect on plant performance, only the estimated effect of subordinate species on the plant performance differed from zero (Figure 1d). Interestingly, ant attendance mostly performed by subordinate species had a negative impact on the mean plant performance (Figure 1d). Therefore, both dominant and subordinate ants can reduce herbivore activity on the plants. However, while dominant ants have a neutral effect on plant reproductive performance, subordinate ants decrease plant reproductive output.

3.1 | Publication bias and data heterogeneity

We found evidence of publication bias in our dataset (Table 1). The intercepts of all Egger's regressions for all *rma.mv* models were negative and different from zero (Table 1). All the models described above exhibited an intermediate level of heterogeneity, explained mainly by the study identity (Table 2).

TABLE 2 Heterogeneity estimates from the models estimating the influence of ant and plant traits on the effect of ant attendance on plants bearing extrafloral nectaries

	I^2 (95% CI)	σ^2 (95% CI)
(a) Overall ant effect on EFN-plants		
Study ID	47.81 (45.89, 49.74)	0.14 (0.08, 0.25)
Plant phylogeny	0.63 (−1.29, 2.55)	<0.001 (0.00, 0.04)
Plant species	<0.001 (−1.92, 1.92)	<0.001 (0.00, 0.02)
Habitat	<0.001 (−1.92, 1.92)	<0.001 (0.00, 0,02)
Total	50.05 (48.13, 51.93)	—
(b) Types of ant effect (herbivorous × plant performance)		
Plant phylogeny	0.41 (−1.15, 1.93)	<0.001 (0.00, 0.03)
Plant species	<0.01 (−1.92, 1.92)	<0.001 (0.00, 0.23)
Habitat	1.12 (−0.79, 3.04)	<0.001 (0.00, 0.13)
Total	46.91 (43.45, 47.29)	—
(c) EFN location*types of ant effect		
Study ID	49.91 (49.00, 51.84)	0.18 (0.89, 0.34)
Plant phylogeny	1.07 (−0.85, 2.99)	0.01 (0.00, 0.11)
Plant Species	<0.001 (−1.90, 1.92)	<0.001 (0.00, 0.03)
Habitat	0.15 (−1.77, 2.07)	0.01 (0.00, 0.19)
Total	51.14 (56.03, 59.87)	—
(d) EFN vascularization*types of ant effects		
Study ID	51.10 (49.18, 53.02)	0.15 (0.08, 0,27)
Plant phylogeny	<0.001 (−1.92, 1.92)	<0.001 (0.00, 0.07)
Plant species	<0.001 (−1.93, 1.92)	<0.001 (0.00, 0.04)
Habitat	0.08 (−1.83, 2.01)	<0.001 (0.00, 0.33)
Total	51.23 (49.31, 53.16)	—
(e) Ant behaviour*types of ant effect		
Study ID	36.77 (34.85, 38.70)	0.10 (0.02, 0.23)
Phylogeny	<0.001 (−1.92, 1.92)	<0.01 (0.00, 0.19)
Plant Species	10.41 (8.49, 12.34)	0.03 (0.00, 0.15)
Habitat	<0.0010 (−1.92, 1.92)	<0.01 (0.00, 0.13)
Total	47.19 (45.27, 49.11)	—

4 | DISCUSSION

By choosing a group of species involved in the same type of protective mutualism and evaluating how some ant and plant traits are related to the effect of ant attendance on EFN-bearing plants, we were able to show that variation in species traits is a key determinant of the outcome in ant-plant protective interactions. Considering together ant effects on herbivores and plant performance, ants have a slight positive effect on EFN-bearing plants. This pattern indicates that most studies investigating the ant protective effect on EFN-bearing plants tend to find that ants provide some benefit to the plants attended by them. But as is expected for generalized facultative mutualisms, there is a substantial variation in the ant effects on plants, and this variability provided insights into the dynamics of this interaction. As we hypothesized, plants bearing EFNs only on reproductive organs and being mainly attended by dominant ant species benefit more from ant attendance than plants bearing EFNs on vegetative organs and mainly attended by subordinate ants. Contrary to our expectation, though, plant species bearing non-vascularized EFNs benefited more from ant attendance than plants bearing vascularized ones. Additionally, the effect of ants on EFN-bearing plants depended on the type of the ant effect evaluated (i.e. on herbivores or on plant performance). While ant effects on herbivores were more constant and positive across different ant and plant traits, ant effects on plant reproductive performance were highly variable and mostly non-different from zero. This pattern raises an important issue because there is a bias in the literature toward studies evaluating the effect of ant attendance on herbivores. It means that our knowledge about these interactions is based mostly on studies reporting the outcomes of an initial subset of processes involved in these interactions. Below, we discussed the ecological and evolutionary implications of our findings to our current knowledge about the functioning of this ant-plant protective mutualism.

4.1 | Magnitude of ant effects on the herbivores and the plant performance

Our results show that ants positively affect EFN-bearing plants, regardless of the plant and ant traits involved in the interaction. However, the effects of ant attendance on herbivore activity are stronger than the ant effects on plant reproductive performance. From an ecological perspective, one could ask why ant effects on plant reproductive performance at the ecological time are weakly positive and highly variable. Two non-mutually exclusive explanations can be the answer to this question. First, there is a potential time lag between the effect of the ants on herbivores and its effect on plant performance. Commonly, plants do not reproduce continuously over time (Fenner, 1998 and references therein). Instead, they produce flowers and fruits in specific periods, with such production being proportional to the energy and nutrients accumulated by the plant over time, including the non-reproductive period (Gendron et al., 2021; Wenk & Falster, 2015). Therefore, it is unlikely that any ant effect on herbivores will translate into an immediate increase in plant reproductive performance. Instead, any ant effect on the plant reproductive performance will be a long-term process resulting from the cumulative effect of ant attendance on plant herbivory over time. Also, the magnitude of such cumulative effect is not constant among EFN-bearing plant species, depending on other plant traits like tolerance mechanisms to certain levels of herbivory (see Tiffin, 2000 and references therein). In this case, the ant effect on herbivory would only translate into increasing plant reproductive performance when herbivory pressure exceeds the plant tolerance threshold, with such threshold varying among plant species.

A second explanation could be the lifespan variation across plant species used in the studies included in our dataset. For long-lived plant species bearing EFNs, any benefit from ant attendance can be expressed over multiple reproductive events, which contrasts with the effect on short-lived (annual species) plants in which any ant effect on herbivores' performance is more prone to be expressed in the immediate reproductive event. Because most studies evaluate the role of ant attendance on plant fitness over very short periods, it may be that they provide more accurate information on ant effect on the performance of short than long-lived plant species. The effect of ant bodyguards on the defence of plants was investigated in other previous meta-analyses (e.g. Chamberlain & Holland, 2009; Rosumek et al., 2009; Trager et al., 2010), with some of them indicating that the protective effect of ants is stronger in long-lived plant species. However, these studies focused on all ant-plant defensive mutualisms (i.e. ant-EFN, ant-domatia and ant-aphid mutualisms), not considering the effect of plant lifespan separately for each interaction type (increasing the chances that the results had been driven by a prevalence of plants with different types of mutualisms in each lifespan group, for example). For this reason, the influence of plant life span on the magnitude of ant bodyguard effects for EFN-bearing plants is a hypothesis that remains to be investigated.

Due to the nature of our data, the mean effects estimated in our analyses may be obtained both because most studies have reported a negligible effect of ants on plants or because positive and negative ant effects we reported in a similar frequency, leading to a neutral mean effect (as explored in the paragraph above). Particularly for the ant effects on plant performance, the average near-zero effect with a marked variation may give evolutionary insights into why EFNs are a labile trait gained and lost many times independently across vascular plants (Weber & Keller, 2013). Over evolutionary time, EFN-bearing plant species have been submitted to different ecological contexts that drove the magnitude of the effects of ant attendance in different directions. Consequently, EFNs should have been mostly favoured in contexts in which ant effects on herbivores have a higher probability of affecting plant performance positively. However, in such contexts in which EFNs can be adaptive, variation in the magnitude in which the herbivory reduction translates into higher plant reproductive performance could lead to variations of the selective pressures acting on EFNs. It can also explain the marked morphological and physiological variation in the EFNs occurring within particular plant lineages (e.g. Marazzi & Sanderson, 2010; Melo, Córdula, et al., 2010; Melo, Machado, et al., 2010, Nogueira et al., 2012, 2013). As more persistent and positive the link between the ant effects on the herbivores and the plant performance, the higher should be the probability of fixation of plant phenotypes with extrafloral nectar traits improving plant attractiveness to the ants in the presence of additive genetic variation in the population.

4.2 | Influence of plant and ant traits on the ant effects on plants

Although we observed that the effects of ants on the performance of EFN-bearing plants tend to be low, we found one exception: ant attendance on plants bearing EFNs exclusively in the reproductive organs largely improved plant reproductive performance.

It indicates that the positive ant effect via protection of flowers and fruits and/or increase in cross-pollination probability (e.g. Altshuler, 1999; Passos & Oliveira, 2004; Sousa-Lopes et al., 2020) surpasses any potential negative effect associated with ant deterrence on pollinators. This result must be discussed with caution since it is based on a small number of studies. However, if biologically meaningful, specific mechanisms may be directing the interaction dynamics between plants and ants when EFNs are exclusively located on reproductive organs. Considering that the spatial distribution of the ants over the EFN-bearing plants is determined by the distribution of EFNs on the plant surface, the higher reproductive performance of plants bearing EFNs on reproductive organs can be a direct consequence of the ants' exclusive attraction to the reproductive organs. Indeed, we observed no effect of ant attendance on the reproductive performance of plants when the EFNs were in both reproductive and vegetative organs. These findings go in the opposite direction of some studies suggesting that extrafloral nectaries could have been favoured over evolutionary time by distracting ants from visiting flowers (e.g. Rosenzweig, 2002; Wagner & Kay, 2002; Villamil et al., 2019). For some plant species, the extension of ant defensive service reducing the negative impact of animals exploiting/predating flowers or fruits could have been the main pressure favouring the maintenance of these secretory structures once in the reproductive organs despite some potential ant-pollinator conflict. Alternatively, while ant effects on vegetative organs will probably require more time to be expressed in a differential reproductive output, ant effects on reproductive organs have a more immediate effect on fruit production. This pattern is expected because there is a temporal decoupling between events of herbivore avoidance in vegetative organs and flower/fruit production in comparison to herbivore deterrence directly on reproductive organs.

Our most surprising result was the relationship between ant benefits and EFN vascularization. The ant effect on herbivores and herbivory was positive and did not differ between plant species bearing vascularized and non-vascularized EFNs. This pattern indicates that ants foraging on vascularized and non-vascularized EFNs are equally effective in attacking potential herbivores that reach the plants. However, the ant effects on plant reproductive performance differed between plant groups, being higher for plant species bearing non-vascularized EFNs, in contrast with our original hypothesis. Since non-vascularized EFNs secrete less nectar than vascularized EFNs (Delgado et al., 2011; Diaz-Castelazo et al., 2005), and because the general benefits of ants to plants are near zero, it may be that, by secreting less nectar, plants bearing non-vascularized EFNs more frequently attain a positive cost–benefit situation. Contrary to what we predicted, non-vascularized EFNs may represent a plant phenotype optimized to have lower nectar costs in particular plant clades (e.g. Nogueira et al., 2012, 2013), maintaining plant benefits when dealing with ant bodyguards. Complementarily, it is important to emphasize the quantitative variation of extrafloral nectaries on plants is a factor co-varying with the degree of vascularization of these secretory structures. Both vascularized and non-vascularized EFNs occur in different abundances and sizes (Nogueira et al., 2012) and multiple plant modules depending on the plant phenology (Nogueira et al., 2020). These two aspects of EFNs have not been explored here due to the absence of quantitative information in the literature. However, even though the effect of ant attendance estimated here for vascularized and non-vascularized plant species can be still modulated by the quantitative variation in the EFNs among species, the effect of EFN vascularization was strong enough to be detected in our models and to be biologically meaningful in the dynamic of these interactions.

Regarding the effect of ant aggressiveness on plants, we found, as expected, that dominant ant species have greater general positive effects on plants than subordinate ant species. In fact, our results showed that ant attendance can even harm plant reproductive performance when subordinate ants mainly attend to the plant. This is interesting because it indicates that behavioural differences in the way ants forage and compete with each other can be a strong mediator of their protective benefits to EFN-bearing plants (Apple & Feener, 2001). The difference in the outcome provided by dominant and subordinate ants can raise some evolutionary implications. For instance, we could expect EFNs to be more strongly favoured over evolutionary time in habitats where plants are more prone to interact with dominant ants (e.g. dry habitats—Leal & Peixoto, 2017). Indeed, there is enough evidence in the literature that in some habitats, such as more seasonal ones, EFN-bearing plants are more likely to interact with dominant ant species, which generates a higher positive outcome for plants (e.g. Leal & Peixoto, 2017; Nogueira et al., 2012, 2015; Passos & Leal, 2019; but see Melati & Leal, 2018). However, it is unclear whether the higher probability of plant attendance by dominant ant species in some habitats is related to EFN traits for ant attraction or a natural difference in the frequency at which dominant and subordinate ants are found to be exploring feeding resources in the vegetation.

5 | CONCLUSIONS

In this study, we found that both plant and ant traits can drive the outcome of ant attendance for EFN-bearing plants. However, these traits influenced the ant effect on herbivory and reproductive performance in different directions and at different magnitudes. This result points to another exciting avenue for investigation, not only of protective ant-plant mutualism dynamics but of mutualistic interactions in general. In addition to the effect of environmental context on the outcome of mutualisms, we now have meta-analytical evidence that trait variation among interacting species plays a marked role in the interaction outcome, and such a role can vary in intricate ways. Since generalized mutualisms are extremely common, the effect we found here is probably pervasive in nature. Finally, because different plant and ant traits affect the plant outcome differently, the selective pressures and evolutionary routes in protective mutualisms may depend not only on the ecological context in which the interaction is embedded but also on the pool of traits exhibited by the species involved in such interactions.

AUTHOR CONTRIBUTIONS

Laura C. Leal designed the study, collected the data, performed the statistical analysis and wrote the first draft of the manuscript. Anselmo Nogueira collected and interpreted the data and revised drafts of the manuscript. Paulo E. C. Peixoto designed the study, interpreted the data and revised drafts of the manuscript. All authors contributed to the final version of the manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

All data and scripts used in the analyses are available in Dryad Digital Repository https://doi.org/10.5061/dryad.5dv41ns90 (Leal et al., 2022).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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