Are seed-dispersing ants elaiosome-limited? An experimental test in a Connecticut forest dominated by myrmecochorous plants

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ARTICLE INFO

ABSTRACT

Food-for-dispersal mutualisms are critical sources of energy flow in ecological networks. By providing a nutrient-rich reward in the form of a fruit or seed to an animal mutualist, plants gain directed dispersal. Myrmecochory is one form of animal-mediated seed dispersal in which ants are recruited to plant seeds by providing a nutrient rich appendage called an elaiosome. It is unclear to what impact nutrients provided by elaiosomes have on ants, and whether the mutualism can tolerate disruptions to this food source. In the Northeastern U.S. mixed deciduous forest ecoregion, a diverse assemblage of herbaceous plants are dispersed by *Aphaenogaster* ants. Since *Aphaenogaster* ants gain a significant nutrient subsidy from this mutualism, it is predicted that loss of these seeds could cause reductions in their population size. To test this hypothesis, I performed a long-term removal of elaiosome food sources for ants. All seeds were removed by hand from three $50m^2$ plots, each paired with control plots with no removal, and a supplementation plot from April to May, from 2010 to 2012. Five years following removal treatments, I collected data on coverage of ant-dispersed plants and the abundance of seed-dispersing *Aphaenogaster* ants. Removal of all sources of elaiosomes resulted in modest reductions in the proportional coverage of ant-dispersed plants relative to control plots. Ant forager abundance was not impacted by the treatments five years after the removal of elaiosomes. In sum, *Aphaenogaster* populations may not always be limited by the availability of elaiosomes or myrmecochorous plants produce can recover quickly (<5 years) to provide enough food for these ants. As long as surrounding populations of understory forest plants are maintained, it appears that both mutualistic ants may rebound from a short-term reduction in the production of elaiosomes.

1. Introduction

Seed dispersal mutualisms are important components of terrestrial food webs since they represent a source of energy flow from plants to animals (Bascompte and Jordano, 2007; Schleuning et al., 2015; Vander Wall et al., 2017). By providing a food source to animals in the form of fruit or seed tissue, plants can achieve directed dispersal to high-quality sites, while animal seed dispersers gain an important, target food source (Silva et al., 1997; Farwig and Berens, 2012). Ant-dispersed plants (i.e. myrmecochores) present a syndrome of traits to recruit ant workers to seeds (Giladi, 2006; Dunn et al., 2007). This plant guild includes a diverse assortment of over 11,000 species that employ this dispersal strategy, and produce diaspores with soft, lipid-rich appendages called elaiosomes (Warren II and Giladi, 2014). Elaiosomes are attractive to ants—foraging workers return the seeds to the parent colony, remove the elaiosomes to feed to brood, and then discard the remaining seed to

ant colony middens (Morales and Heithaus, 1998). Elaiosomes contain multiple nutrients that can be limiting for ant colony development, including fats and amino acids (Fischer et al., 2005). High abundance of both ant-dispersed plants and ants in some habitats suggest this is an important limiting food source for ant populations (Ness et al., 2009). Evidence for elaiosomes as a source of limiting nutrients originates from studies demonstrating that the nutritional needs of developing ant larvae are supplemented by the lipids in elaiosomes (Fischer et al., 2008). Consequently, elaiosome-bearing plants could have trophic impacts on ants by increasing number of workers or new colonies when ant populations are food-limited. However, tests of this "elaiosome-limitation hypothesis" are rare (but see Warren II et al., 2019). Mechanistic work on the nutritional benefits of elaiosomes have focused on ant colony traits or fitness (e.g. Bono and Heithaus, 2002; Gammans et al., 2005), rather than ant population-level abundance in field conditions.

Myrmecochory is common in temperate forest understory plants for

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spring-ephemeral and summer-ephemeral life history strategies (Beattie and Culver, 1981; Boulay et al., 2007). Spring ephemerals have short leafing and flowering times, often senescing before the forest canopy closes. In eastern North American forest habitats, *Aphaenogaster* ants are often numerically dominant, and evidence suggests these ants are the most effective dispersal mutualist for myrmecochores. *Aphaenogaster* ants rapidly discover and move diaspores (Smith et al., 1989), which prevents seed predation (Heithaus, 1981; Ruhren and Dudash, 1996). Given the ubiquity of *Apheanogaster* in deciduous forests of in eastern North America, the "elaiosome-limitation hypothesis" has been primarily tested in this system (e.g., Warren II et al., 2019). In particular, elaiosomes as a supplemental food source for *Aphaenogaster* colonies and can be important when insect prey is less available (Clark and King, 2012). While *Aphaenogaster* are specialized as seed dispersing ants, it is less clear to what degree this is an obligate mutualism limiting their populations.

Demonstrating whether or not seed dispersing ants are limited by the availability of elaiosomes is an important objective for the protection of ant-dispersed plant communities. Given the specificity of this mutualism, there is concern that habitat disturbance and fragmentation could negatively impact myrmecochorous communities or their seed-dispersing ants like *Aphaenogaster* (Ness and Morin, 2008; Parker et al., 2021). Myrmecochores in these systems are predominantly spring ephemerals and seasonal greens that can dominate the herbaceous plant layer in intact closed-canopy mixed-deciduous forests (Handel et al., 1981). However, in smaller forest fragments, edge effects might drive reduced abundance of myrmecochores and seed-dispersing ants (Ness and Morin, 2008). With the loss of these food sources due to habitat fragmentation, there could be cascading effects driving decline of this putative mutualist ant *Aphaenogaster*.

The goal of this study was two-fold: First, to describe the diversity of ant-dispersed plants in the understory of Connecticut forests. In the study area of this experiment (New Haven, Hartford, and Litchfield Co., Connecticut, USA), there is not a comprehensive list of ant-dispersed plants nor data on the relative abundance of these plants in forest understories. Second, since long-term data on ant-myrmecochore interactions are limited (Heinken and Winkler, 2009), I tested the "elaiosome-limitation hypothesis" in an environment in which myrmecochores dominate. Food limitation for populations is often context dependent, so this study addresses loss of elaiosomes for a short-time period in one single habitat type. In this specific location, I predicted that locations in which myrmecochores were exceptionally abundant would represent areas where the carrying capacity of *Aphaenogaster* could be manipulated if this pulse of nutrients was consistently found at high frequency. In all, there are relatively few studies indicating the importance of elaiosomes for populations of Aphaenogaster, particularly in situations where this food source is not available for more than one growing season.

2. Material and methods

2.1. Surveys and description of Connecticut ant-dispersed plants

I reviewed the literature on herbaceous myrmecochorous plants, searching for species with known records in Connecticut forests that are native, non-woody, non-graminoid plants. I queried Web of Science with the search terms "myrmecochor*", "elaiosome*", and "ant" + "dispersed" + "plant". Reviewing the abstract, I verified if the study was about ant-dispersed plants and created a list of species found in studies conducted in eastern North America. With this species list, I used online references, including the New England region maps on GoBotany.com (Native Plant Trust, 2021), to see which plant species had distributions including any Connecticut counties. Connecticut myrmecochores were included in Table 1 if they met one of two criteria: ant dispersal was previously validated through behavioral observation of seed removal (e.g. Gaddy, 1986) or nutritional assays in which ants

Table 1
List of ant-dispersed plants found in Connecticut forests.

Plant species	Common Name	Ant-dispersal reported	At Henry Buck Plot
Anemone americana[a]	Roundlobe hepatica	Warren and Bradford, 2014	X
Anemone quinquefolia	Wood anenome	Beattie and Culver, 1981	X
Asarum canadense	Canadian wild ginger	Smith et al., 1989	X
Claytonia caroliniana[b]	Carolina springbeauty	Miller and Chambers, 2006	
Claytonia virginica	Eastern springbeauty	Handel et al., 1981	X
Corydalis flavula[c]	Yellow fumewort	Beattie and Culver, 1981	
Dicentra canadensis	Squirrel corn	Thompson, 1981	X
Dicentra cucullaria	Dutchman's breeches	Handel et al., 1981	X
Erythronium americanum	Trout lily	Ruhren and Dudash, 1996	X
Galium circaezans	Forest licorice	Gaddy, 1986	
Hepatica nobilis[d]	Sharplobe hepatica	Handel et al., 1981	
Melampyrum lineare[c]	American cow-wheat	Gibson, 1993	
Sanguinaria canadensis	Bloodroot	Heithaus, 1981	X
Trillium cernuum[b]	Nodding trillium	Gunther and Lanza, 1989	
Trillium erectum	Red trillium	Gunther and Lanza, 1989	X
Trillium grandiflorum	Great white trillium	Gunther and Lanza, 1989	
Trillium undulatum	Painted trillium	Gunther and Lanza, 1989	X
Uvularia grandiflora	Large-flowered bellwort	Robertson, 1897	
Uvularia perfoliata	Perfoliate bellwort	Beattie and Culver, 1981	
Viola blanda	Sweet white violet	Culver and Beattie, 1978	X
Viola canadensis	Canada violet	Beattie and Lyons, 1975	
Viola palmata	Wood violet	Culver and Beattie, 1978	
Viola pedata	Birdfood violet	Culver and Beattie, 1978	
Viola pubescens	Downy yellow violet	Culver and Beattie, 1978	X
Viola rostrata	Long-spurred violet	Culver and Beattie, 1978	
Viola sororia	Common blue violet	Culver and Beattie, 1978	X

List generated from field surveys in Connecticut forests and literature review of empirical work on myrmecochory in the eastern U.S. Status of verified seed dispersal syndrome highlighted in each row, along with description of observation status. *Corydalis sempervirens*, *Dicentra canadensis*, *Trillum cernuum*, and *Viola rotundifolia* are putatively ant-dispersed and found in Connecticut counties, but no empirical evidence was found demonstrating ant recruitment to seeds.

[a] Taxonomic status has been revised since publication describing *Anenome americana*, new revisions place this species as *Hepatica nobilis* var. *obtusa*.
[b] Elaiosome traits reported for all relatives in this genus.
[c] These species have an annual life history, all other species are perennials.
[d] Taxonomic status has undergone significant revisions since original publication which described *Hepatica acutiloba*, sharplobe hepatica now listed as *Hepatica nobilis* var. *acuta*).

consumed elaiosomes (e.g. Bono and Heithaus, 2002). In two cases plant species in a shared species complex were included as all other members of that genus or species complex were also ant-dispersed, but this stipulation is denoted in Table 1. All herbaceous plants I observed in Connecticut during transect surveys were also checked for evidence of ant-mediated seed dispersal following the same literature review criteria. Current scientific names and life history (annual or perennial) of all plant species verified using USDA PLANTS database (USDA and NRCS,

2022). Additionally, plants seen during surveys or while collecting seeds for Clark and King (2012) were checked within the literature. These species lists were also checked following the same approach as the literature review. To be added to Table 1, evidence of behavioral observation or feeding were necessary (ranging from Robertson, 1897 to Warren and Bradford, 2014). The genus *Viola* has been systematically revised since most reports of ant dispersal were published (e.g., Culver and Beattie, 1978). Thus, *Viola* reported in Table 1 were updated to reflect the most current names in the New England region reported through GoBotany.com (Native Plant Trust, 2021).

In 2009–2010, I completed four surveys on ant-dispersed plant abundance in secondary forests. The goal of these surveys was to provide preliminary data on the most common species found across three different classes of secondary forests typical to the state of Connecticut (each >20 km from each other). To test the elaiosome-limitation hypothesis, I sought to find a location in which ant-dispersed plants numerically dominate the understory plant community. At each site, I designated transects starting at the habitat edge and continued inward to the center of the forest fragment. A meter tape was laid down across the forest floor and pinned with a tent stake at both ends. Plants overhanging this line were quantified by measuring the total coverage over our transect lines in cm. Sampling effort varied among sites, for more details on the size of transects, see Figs. S1-S5. All non-graminoid, herbaceous, vascular plants were identified species, including both ant-dispersed and non-ant dispersed plants.

2.2. Simulating loss of elaiosomes at Henry Buck Trail

I employed a manipulative experiment to remove all sources of elaiosomes in plots arranged in a 3×3 factorial design. There were three replicates for each treatment making up nine plots at 50m^2 each (Fig. S1). I chose to conduct the habitat manipulation at the site where the highest species richness of myrmecochores were present (Henry Buck Trail, Fig. S2). In 2010, 2011, and 2012, I removed all potential sources of elaiosomes that could be provided to the ant community. I removed the following by hand: developing flower heads of all apparent myrmecochores, visible flowers, developing seed pods, and entire diaspores. Removal took place in April–May each year (one visit each month) to ensure all members of this plant guild could be manipulated. Given density estimates of April 2010 preliminary transects, for removal treatment, I removed >155,000 flowers or seed pods over the three-year manipulation. In a third positive treatment, I supplemented plots with all *Trillium* seed pods that were extracted from removal treatments immediately following completion of each block on the same day. *Trillium* has large, apparent seeds in which ants remove elaiosomes (personal observations, Gunther and Lanza, 1989). *Trillium* supplementation was used as a complimentary test of the elaiosome limitation hypothesis. I predicted that a supplementation of a single myrmecochore species can impact *Aphaenogaster* populations or foraging activity.

Three years of elaiosomes removal deprived colonies located within these transects for a long-term period. The time period represents a long-term exclusion of this nutritional resource, and it would be difficult for *Aphaenogaster* in these plots to collect many elaiosomes outside the manipulated area given that their 0.67 to 1.6 m^2 foraging territory is smaller than the plots (Weseloh, 1994; Lubertazzi, 2012). Furthermore, many ant-dispersed plants have restricted dispersal distance, and while recolonization can occur, it is typically at a slow rate (Andersen, 1988; Sasidharan and Venkatesan, 2019). The eight-year period (three-year removal, five-year sampling delay) represents the time in which new colonies could be produced and develop given that *Aphaenogaster* colonies have a median lifespan of eight years, and new colony development could take up to two years (Lubertazzi, 2012).

To assess ant population response to changes in elaiosome availability, I measured the colony-level foraging activity of *Aphaenogaster* and other ground-foraging ants on May 15, 2017 (*Aphaenogaster* colony sampling method modified from Lubertazzi, 2012, Mitchell et al., 2002).

In this assay, five cookie baits (pecan sandies) were placed within each of the larger sub-plots and left for two hours in mid-day (Fig. S6). One bait was placed in the center of each plot, while the other four baits were placed in the corner 2 m from the plot border. After the 2-h waiting period, I returned to count and identify ant species with workers actively taking pieces of bait. If multiple ants were collecting baits and moving in a single column, I followed workers to a nest entrance to verify them as representatives of a single, shared colony. A single worker of a one species was also counted as representing a single unique colony.

To evaluate the reduction in elaiosome availability in response to my removal and addition manipulations, I completed a complete plant-community survey in May 2017, using line transect sampling at each sub-plot (Fig. S6). Two line transects were laid diagonally in each plot, making a total transect length of 140 m. This method gave detailed total coverage of every ant-dispersed plant species and all other plant species (pooled together as "non-ant-dispersed plants").

2.3. Statistical methods

Three approaches were used for statistical analysis in R version 4.0.5 (R working group, 2021). To quantify the impact of treatments (i.e., experimental removal or addition of sources of elaiosomes), I employed an analysis of proportional plant coverage at Henry Buck. This was fitted as a binomial Generalized Linear Mixed Model (GLMM) using the lme4 package (Bates et al., 2015). Model estimates, such as predicted mean, standard error, and post-hoc tests, were extracted using the emmeans package (Lenth, 2016). *P*-values for fixed effects were calculated using the car package (Fox and Weisberg, 2011). To evaluate the impact of treatments on *Trillium* coverage, I ran one follow up GLMM using the same model specifications, but with total coverage of *Trillium* fitted to a negative binomial distribution. All GLMMs used block as a random effect and elaiosome manipulation treatment as a fixed effect. To evaluate treatment effects on ant colony abundance and *Trillium* abundance, a Kruskal-Wallis rank test was employed due to the highly non-normal structure of these data (Ruxton and Beauchamp, 2008). For analysis of species richness (estimated site-specific rarefaction), I used the vegan package (Osksanen et al., 2019). Estimates of diversity in the plant species pool used *Chao1* index to make comparisons across sites with varying sampling effort (e.g. Dilworth et al., 2021).

3. Results

My literature and field surveys revealed there are 25 records of native, herbaceous, ant-dispersed plants following our search criteria. These are predominantly native spring ephemerals found in the region's mixed-deciduous forests, and with the exception of *Corydalis flavula* and *Melampyrum lineare*, all are perennial wildflowers. The species richness and composition of the plant communities surveyed is reported in supplemental tables and Figs. S2-S5, with Trout lily *(Erythronium americanum)* being the only myrmecochore species found at all sites. Notably, line transects revealed that the highest species richness of ant-dispersed plants was observed at the Henry Buck Trail at American Legion State Forest in Barkhamsted, CT (Fig. S2). Myrmecochore species richness and coverage was noticeably higher than estimates from larger region-wide surveys (Warren II et al., 2021). Thirteen myrmecochore species occurred in this site's transect and the site had a percent coverage for myrmecochores of 87.2% within the herbaceous layer.

My post-manipulation plant community surveys at Henry Buck trail demonstrate ant-dispersed plants still dominated the proportional cover of the understory, herbaceous layer. Dutchman's breeches (*Dicentra cucullaria*), Red trillium (*Trillium erectum*), Spring beauty (*Claytonia virginica*), and Trout Lily (*Erythronium americanum*) were the four most abundant ant-dispersed plants at the end of this survey (Fig. 1). Hand-removal treatments had a modest reduction in the proportional coverage of ant dispersed plants, but this difference was indeed significantly different (Fig. 2, Binomial GLMM, $\chi^2 = 16.85$, df = 2, $P < 0.001$).
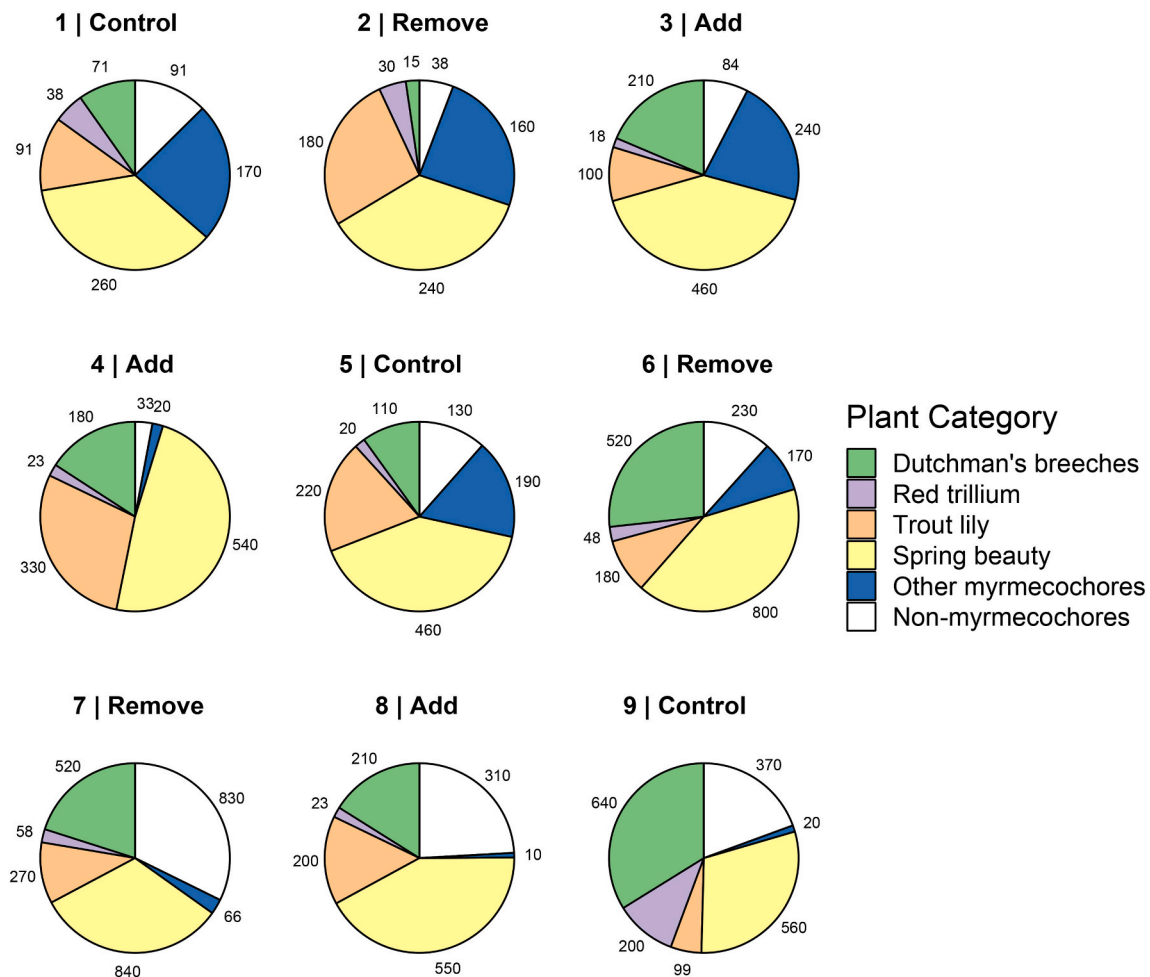
Fig. 1. Final plant community survey results and subplot layout for Henry Buck Trail plots. Each circle represents total plant coverage based on transects among nine 50m^2 plots. Circle sections represent the proportional abundance of plants in each category: four common myrmecochore species (*Claytonia virginica*, *Dicentra cucullaria*, *Erythronium americanum*, *Trillium erectum*), other myrmecochores, and all non-myrmecochore herbaceous plants. Each labeled pie-chart represents a subplot and manipulation treatment. Total plant coverage in linear transects were measured to cm (indicated by values above each pie section).

Control plots with no manipulation had the highest proportional coverage of ant-dispersed plants (Fig. 2, Tukey HSD). In terms of magnitude of this effect, 73.8% of the transects in control plots were covered by ant-dispersed plants, compared to 67.3% in removal plots, indicating they were still dominant in terms of leaf area. *Trillium* supplementation plots did not result in increased numbers of *Trillium* plants compared to controls (Negative binomial GLMM, posthoc test, Z ratio = −2.00, P = 0.112). Similarly, *Trillium* supplementations plots did not have significant more *Trillium* plants compared to removal plots either (Negative binomial GLMM, posthoc test, Z ratio = −1.46, P = 0.309). Plots with addition of *Trillium* seeds to plots had intermediate proportional covered by ant-dispersed plants overall (Fig. 2, Tukey HSD). In all, these results suggest that the manipulation only moderately reduced the proportional abundance of myrmecochores and they remained dominant over non-myrmecochores with respect to herbaceous plant cover (Fig. 2).

Surveys of the ground-foraging ant community and *Aphaenogaster* colony abundance yielded five species of ants, including *Aphaenogaster rudis* group, *Camponotus pennsylvanicus*, *Lasius neoniger*, *Myrmica punctiventris*, and *Tapinoma sessile*. With respect to timing, this baiting assay took place six years after the last manipulation of all sources of elaiosomes in removal treatments. I observed no difference in the abundance of seed-dispersing *Aphaenogaster* colony abundance among removal, control, or supplementation treatments (Fig. 3, Kruskal-Wallis, χ^2 = 1.15, df = 2, P = 0.56). In the control treatments 1.33 (SE 0.25)

Aphaenogaster colonies were observed, compared to 1.53 (SE 0.16) in the removal, and 1.26 (SE 0.26) in the supplementation. Additionally, there was no difference among treatments in the recruitment of non-*Aphaenogaster* ant colonies to these baits (Kruskal-Wallis, χ^2 = 0.78, df = 2, P = 0.67). For non-*Aphaenogaster* ants, 2 (SE 0.74) colonies were observed in the control treatments, 2.53 (SE 0.85) in the removal, and 2.2 (SE 0.67) in the supplementation.

4. Discussion

The impact of anthropogenic disturbance disrupts dispersal mutualisms (Markl et al., 2012). Specialized mutualisms are particularly susceptible to extinctions because if one partner is extirpated from an environment, the mutualistic partner risks demographic failure (Farwig and Berens, 2012). My results indicate that in intact forests ant-myrmecochore mutualisms might be able to tolerate loss of elaiosome-bearing seeds for a short period of time. If elaiosomes are not produced for three years, myrmecochores were still able to persist as dominant members of the understory herbaceous plant community and *Aphaenogaster* were still active in both removal and control plots. This ability of myrmecochores to persist following this disturbance may be explained by the dominance of perennial myrmecochores in this community since only two species were found to be annuals. Conversely, large-scale habitat changes may require longer time scales for recovery of ant-dispersed plants (Sorrells and Warren II, 2011). Climate may also
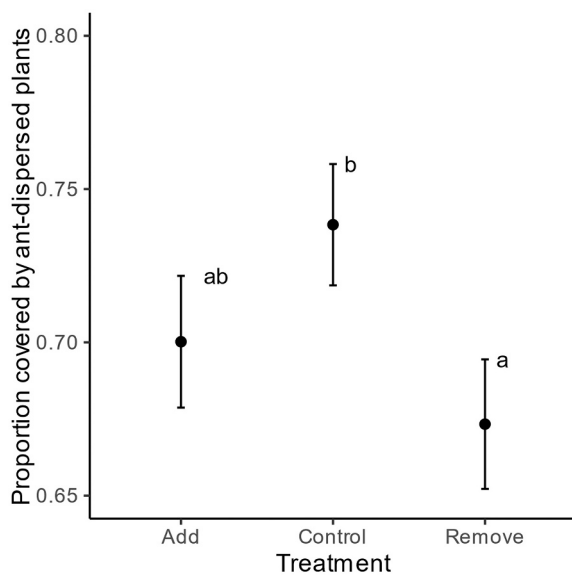
Fig. 2. Plot for binomial GLMM of proportional ant-dispersed plant cover (cm) out of all non-woody understory plants sampled. Letters indicate post-hoc tests (Tukey HSD) across all treatment groups. Points indicate the estimated marginal means from the binomial GLMM, and their associated error terms are represented by error bars.
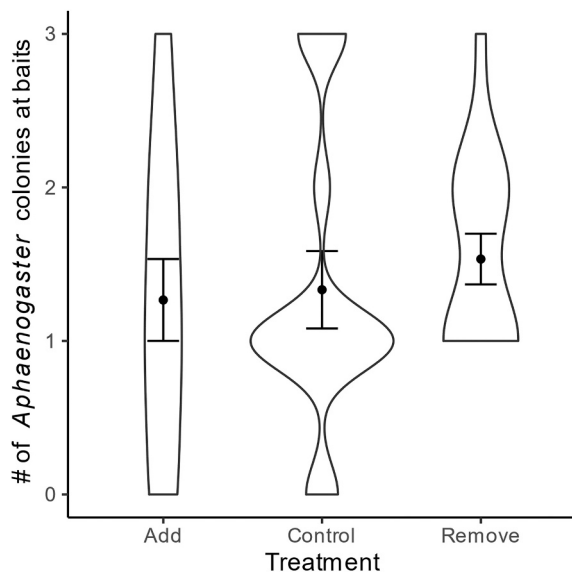

Fig. 3. Violin plot for colony abundance among three treatments. Colony abundance is measured as the number of unique *Aphaenogaster* colonies with workers visiting baits in the 2017 assay. Width of violin plots within each treatment indicates the relative number of counts of that number, while length indicates the range of observed outcomes (0–3 colonies per bait). Center points and error bars indicate mean and standard error of the mean.

impact mutualistic interactions, as different ant-dispersed plants can stagger seed release throughout the season to avoid competition (Warren II et al., 2014). The phenological pattern of myrmecochores could be disrupted by climate change if seed release and ant activity become asynchronous (Warren II et al., 2011). Our experiment did not entirely remove all seeds from the habitat, only small plots. At a larger scale, depleted seed banks can severely limit recovery for herbaceous plants (Lang and Halpern, 2007). Seed depletion may be slower to recover from given some reports of high seed-predation rates for myrmecochores in the U.S.'s eastern deciduous forests (Heithaus, 1981).

Contrary to the predictions of the elaiosome-limitation hypothesis, I did not observe evidence of a population-level impact of elaiosome removal on *Aphaenogaster* foraging activity. I observed no change in the recruitment of *Aphaenogaster* workers to baits during our sampling process, matching findings from in which removal of elaiosome-bearing plants did not reduce *Aphaenogaster* worker abundance or nest occupancy (Warren II et al., 2019). In other systems, seed-dispersing ants can be limited by other resources other than elaiosomes. For example, surveys show that *Aphaenogaster* are probably not elaiosome-limited in small forest fragments (Mitchell et al., 2002). However, my results should be interpreted carefully as the study area only encompasses a single population of *Aphaenogaster* colonies and the study lacks data on *Aphaenogaster* abundance prior to elaiosome removal. *Aphaenogaster rudis* group ants form ephemeral nests in spring and summer, moving to more permanent locations to overwinter (Lubertazzi, 2012), thus it is possible that over long time periods colonies located near the border of experimental plots could move out of the removal area. Additionally, elaiosomes' nutrients may provide other resources to ants that are important for performance outside colony size or density, such as the production of winged alates (i.e., reproductive castes). While closer analysis of nutrition acquisition from elaiosomes show weak impacts on reproduction or sex allocation in colonies of seed-dispersing ants (Caut et al., 2013), other work on *Aphaeanogaster* collected from Connecticut forests show elaiosomes could be an important source of nutrients for brood production by queens (Clark and King, 2012).

My experiment is limited due to the geographic focus on a single forest system, and the patterns observed may be different in other forest fragments. I intentionally chose a site where myrmecochores were abundant to test this hypothesis, but the impacts on *Aphaenogaster* for myrmecochore removal might be more severe in marginal habitats where myrmecochores are comparatively rare members of the understory plant community. For example, *Aphaenogaster* are less common on the edge of forest fragments as well as some of their plant mutualists (Ness and Morin, 2008). Like in other seed-dispersal mutualisms, reduced abundance of *Aphaenogaster* may be the result of habitat fragmentation and competition from invasive species (Rodriguez-Cabal et al., 2012; Meadley Dunphy et al., 2016). Finally, the experiment performed here could be improved with a longer-term removal and sampling regime in which the response to elaiosome loss is measured with starting conditions of myrmecochore and *Aphaenogaster* populations. For our myrmecochore species found in surveys, twenty-three out of twenty-five were perennial plants, including all species of *Trillium*, meaning a longer removal period of seeds may be required to significantly reduce the populations of mature plants.

Finally, several factors may explain why we saw no effects of manual elaiosome removal on *Aphaenogaster* foraging activity. *Aphaenogaster* may have recovered quickly following the three-year removal, returning to prior numbers for the multiple growing seasons occurring between removal and the bait assay. Other conditions may be site-specific. For example, *Aphaenogaster* potentially benefit most from elaiosomes when insect prey can be scarce (Clark and King, 2012). However, at this site insect prey may not be limiting at all. At other locations where insect prey is less available, there could be a significant population-level impact of elaiosomes. But this gap in food availability perhaps did not occur at the field site selected. Furthermore, the nutritional benefits of elaiosomes vary greatly among plant species (Pfeiffer et al., 2010) and our site may have included myrmecochores that provide a relatively low reward. Lower relatively nutritional quality of elaiosomes may explain why removal had a minimal impact on *Aphaenogaster*. It is possible that elaiosomes of higher quality may be provided by a different plant-community composition than the one observed at the site this experiment was conducted.

5. Conclusions

In order to understand seed dispersal ecology, it is critical to

establish the benefits both dispersers and plants receive from the interaction. In plants, ant-mediated seed-dispersal syndrome shows clear specialization for recruiting and attracting ants. Plants gain wide-ranging fitness benefits, including mechanisms like protection from natural enemies or movement to high-quality microsites. *Aphaenogaster rudis* are often the most effective ant species with regard to successful seed dispersal of many myrmecochore species (Ness et al., 2009). Conversely, there is less evidence that *Aphaenogaster* or other seed-dispersing ants are participating in a mutualism. For ants, myrme-cochory may vary from mutualism, to commensalism, even to parasitism (Warren II et al., 2019). It appears likely that *Aphaenogaster* are dependent on elaiosomes only under certain environmental contexts (Clark and King, 2012). With regards to the conservation of forest un-derstory plants and spring ephemerals, it is encouraging to know that gaps in the availability of elaiosomes are unlikely to negatively impact *Aphaenogaster* populations alone. Recent large-scale surveys in eastern US forests showed ant-dispersed understory plants are not necessarily more dispersal limited than other dispersal modes (Warren II et al., 2021). While elaiosomes represent a large pulse of nutrients for ants represented by a diversity of species found in the understory of Con-necticut forests, this seed dispersal service provided by ants is resilient to short-term reductions in elaiosome production. Further work should investigate how changes to climate or large-scale disturbances might negatively impact these seed-dispersal mutualisms in forest fragments.

Declaration of Competing Interest

The author has no competing interests to declare.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fooweb.2022.e00242.

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