Title

An ant-dispersed plant community recovers following a small-scale disturbance in a Connecticut forest

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Abstract:

Many species of plants exhibit a dispersal syndrome called myrmecochory, in which nutrient-rich seed appendages recruit ants which in turn transport seeds. This food-for-dispersal mutualism is common in the understory spring ephemeral and seasonal-green plant community found in temperate deciduous forests of Eastern North America and Europe. In the Northeastern U.S. mixed deciduous forest ecoregion, common wildflower genera including *Trillium, Erythronium, Dicentra, Claytonia* and *Hepatica* are dispersed by the facultative mutualist ants *Aphaenogaster.* Current evidence suggests that this mutualistic ant-plant complex depends on intact, closed-canopy forest and that the loss of one mutualistic partner could cause the decline of the other. It is unclear if, following disturbance, this mutualistic community can recover and support typical abundances of either ants or plants. Forests in Connecticut (USA) are particularly impacted by urbanized development, but the myrmecochores that could be impacted by this land used changes are not well described systematically. To address this shortcoming, I sampled multiple locations where myrmecochores were common. At a particularly intact site, I performed a long-term, small-scale removal of all elaiosomes-bearing diaspores in a gridded plot in a forest understory dominated by myrmecochores. From April to May from 2010 to 2012, all seeds were removed by hand from three 50m2 plots, each paired with control plots with no removal. Following predictions of the life history of plants and ants in this system, I returned in Summer 2017 to collect data on proportional coverage of ant-dispersed plants and the abundance of seed-dispersing ants relative to the entire ant community. I found that despite entirely removing three growing seasons worth of elaiosomes, there were modest reductions in the proportional coverage of ant-dispersed plants. Ant communities were not impacted or had made a recovery within the 6-year duration of the experiment. Consequently, I conclude that as long as surrounding closed canopy secondary forests are maintained, mutualistic ants in ant-dispersed plant communities have a robust ability to rebound from a reduction in this food source.

Introduction

Seed dispersal mutualisms are important components of terrestrial food webs since they represent a source of energy flow from plants to animals. By providing a food source to animals in the form of fruit or seed tissue, plants can achieve directed dispersal to high-quality sites. Many seed-dispersal mutualisms exhibit some degree of specialization, in which there is a co-evolutionary history between animal disperser and the dispersed plant species. In the case of myrmecochorous plants, or ant-dispersed plants, some forest understory plants present a syndrome of traits associated with seed dispersal by generalist ants. Plants with this dispersal strategy produce diaspores with fleshy, lipid-rich appendages called elaiosomes. Elaiosomes are particularly attractive to ants, and 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. Elaiosomes contain multiple nutrients that can be limiting for ant colony development, including fats and amino acids. High abundance of both ant-dispersed plants and ants in some habitats suggest this is an important limiting food source for ant colonies that increase ant population size. However, tests of this “dispersal mutualist limitation hypothesis” have varied across systems.

Myrmecochory is common in temperate forest understory plants following the spring ephemeral life history strategy and observed in North American and Eurasian deciduous forests (Gorb and Gorb citation). These relatively small flowering plants have short leafing and flowering times, often completing emergence, pollination, and seed production before the forest canopy closes. In North American habitats, *Aphaenogaster* ants are numerically dominant, and evidence suggests these are the most effective dispersal mutualist for myrmecochores. *Aphaenogaster* ants rapidly discover and move diaspores, preventing seed predation by small mammals. Given the ubiquity of these ants, the “dispersal mutualist limitation hypothesis” has been primarily tested in this system (e.g. Warren et al. 2019). Under lab conditions, elaiosomes are a food source for *Aphaenogaster* colonies and can be particularly important when another insect prey is not available (Clark and King, 2012).

Given the specificity of this mutualism, there is concern that habitat modification, disturbance, or fragmentation could negatively impact myrmecochorous communities or their seed-dispersing ants like *Aphaenogaster*. It is predicted that disturbance which removes one of the mutualistic partners could negatively impact populations of the other. Consequently, there could be cascading effects as the result of the decline of this putative mutualist ant *Aphaenogaster*. In areas where ant-dispersed plants would otherwise dominate, removal of this mutualist could negatively impact *Aphaenogaster* populations if they are food limited.

Methods:

*Surveys and description of Connecticut ant-dispersed plants*

In 2009-2010, I completed a range of surveys with volunteer students from Central Connecticut State University and University of Connecticut on ant-dispersed plant abundance in secondary forests. The locations, although not exhaustive, represent sites that were accessible and had reports from amateurs about high ant-dispersed plant abundance. The goal of these surveys was to provide preliminary data on the most common species found across a range of secondary forests typical to the state of Connecticut, and to find a location in which ant-dispersed plants numerically dominate the understory plant community. In order to test the hypothesis that mutualistic ants are limited by elaiosomes, it should be the case that high population densities of ants are found in areas where these are dense. Consequently, also, manipulation of the elaiosomes abundance should be expected to reduce the density of foraging ants or colony abundance.

*Small-scale disturbance trails at Henry Buck Trail*

We tested how small-scale disturbance to elaiosomes-bearing plants would impact the abundance of *Aphaenogaster* in an extremely high-density scenario. In a Connecticut forest, we designated plot in which ant-dispersed plants form 90% of the coverage of understory, non-woody plants and represent most of the diversity of herbs (data from supplemental figures?). In 2010, 2011, and 2012, I removed all apparent seedpods and flowers of ant-dispersed plant species in April-May each year (one visit each month). Given density estimates of April 2010 preliminary transects, we harvested over xxxx amount of flowers or seed pods (can I calculate this somehow?).

*Statistical methods:*

Three approaches were used to statistical analysis in R version 4.0.5 (R working group, 2021). For analysis of proportional plant coverage at Henry Buck, I constructed a Generalized Linear Mixed Model (GLMM) using the lme4 package (Bates et al. 2015), and model estimates 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 treatment effects on ant colony abundance, as measured by recruitment to baits, 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).

Results:

The composition of the plant communities surveyed is reported in supplemental tables and figures S1-Sx. *Erythronium americanum*, *Trillium erectum*, *Dicentra cucularia*, and *Claytonia virginia* were found to be common. We chose to the habitat manipulation at a site where all members of the myrmecochore complex easily found in Connecticut were present.

Spring 2017 surveys of the plant community reveal a significant impact of historical removal on the proportional coverage of ant-dispersed plants in plots (Binomial GLMM, χ2 = 16.85, df = 2, *P* < 0.001). 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. Plots with addition of *Trillium* seeds to plots had intermediate proportional covered by ant-dispersed plants (Fig. 2, Tukey HSD).

2017 baits used to record 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*. This sample event following three-years of myrmecochore removal and a six-year waiting period. We observed no difference in the abundance of seed-dispersing *Aphaenogaster* colony abundance among removal, control, or supplementation treatments (Fig. 3, Kruskal-Wallis, χ2 = 1.15, df = 2, *P* = 0.56). *Aphanogaster* workers were present at every observed bait in the removal treatment, additionally indicating they were abundant in these plots. Additionally, there was no difference among treatments in the recruitment of non-*Aphaenogaster* ants to these baits (Fig. 3, Kruskal-Wallis, χ2 = 0.78, df = 2, *P* = 0.67).

Discussion:

Acknowledgements: I thank the staff at American Legion State Forest (Connecticut Department of Energy and Environmental Protection, Parks Division) for allowing me to tag and manipulate plants in Barkhampsted, CT. Josh King (University of Central Florida) provided guidance on the experimental plot layout. Multiple volunteers contributed to the laborious removal experiments, including Melissa Bernardo, Chris Berthiaume, Megan Clark, Joe McLaughlin, and Lauren Verner. Taiga Araki assisted with ant baits and ant-dispersed plant coverage surveys. Chad Seewagen (Great Hollow) and Vera Pfeiffer (Washington State University) provided helpful feedback on revisions of this manuscript.

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Figure captions:

Fig. 1. Experimental design for Henry Buck Trail plots. Nine 50m2 plots were laid out in Spring 2009 and marked with GPS waypoints coordinates and flagged at corners. Arrow indicates cardinal orientation, and each panel describes the factorial treatment design. The asterisk indicates a single plant in which the overstory was dominated by *Pinus strobus* (White Pine) rather than a deciduous canopy.

Fig 2. Plot for binomial GLMM of % ant-dispersed plant cover out of all non-woody understory plants sampled (treatment as fixed effect, meter transects as random effects). 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 recruitment lines of workers found at baits in 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.

Fig. 1.

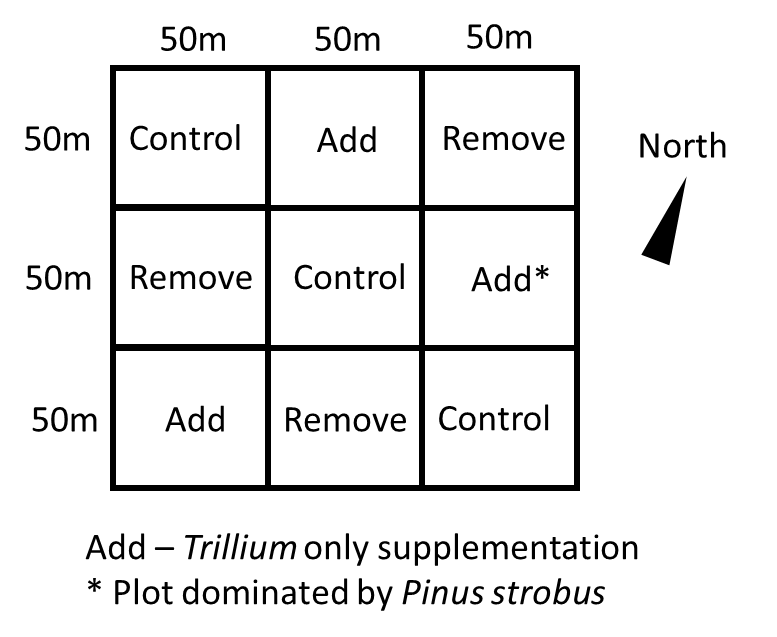


Fig 2.



Fig. 3.

