**Response for review**

Thanks to the reviewer and handling editor for detailed comments. I have addressed all the changes in my detailed response to review below. Editor and reviewer comments are in standard font, **my responses are bold**, ***and edited text quoted from the manuscript is in bold and italics.***

**Detailed notes on response to review:**

Associate Editor:  Two excellent reviews have been completed, and both reviewers found that the manuscript had potential, but was lacking considerable methodological detail relating to sampling frequency and protocol.  The paper was limited in its ability to test the “elaiosome-limitation hypothesis” by the fact that “after” analyses of plant cover were not tied statistically to the original communities present at the site. *If possible, paired analyses of plant communities at each site would strengthen the paper.* I agree with reviewer 2 that some clarification is needed about the biology of the elaiosome-bearing plants (particularly whether they are perennial or annual, and how plant populations might be expected to change over the relevant timescale with this in mind). *I also concur that the writing and synthesis in the discussion should improve.* I would invest considerable effort in clarifying how this study complements and builds on Warren et al. 2019.

**Thank you for your comments and the summary of reviewer feedback. I have focused my efforts on improving the methodology sections, adding significant text throughout this section of the manuscript. I have also updated the discussion to be more careful in the interpretations and followed reviewer suggestions on how to cautiously interpret this test of the elaiosome limitation hypothesis. I addressed the limitations of the “before” and “after” plant and ant community data in detail in response to reviewer #1. Thanks to the very precise suggestions of both reviewers, I believe this manuscript is now much stronger and draws its conclusions more carefully in the discussion, abstract, and results sections.**  
  
I can’t find Warren et al 2018 in the ref list.  Do you mean Warren et al. 2019?

**Change made. Apologies for the mistake, this is an instance where the early-online publication was made available in late 2018 and then appeared in print in Ecological Entomology in early 2019. All references point to the 2019 citation now.**

**Given the importance of this citation on the hypothesis and conclusion, I have followed the suggestion to ensure the text compliments Warren et al. 2019. Addressing many of the reviewer comments aided in this effort. In addition, I added a section to 5. Conclusions providing more detail to highlight this connection to Warren et al. 2019. I reviewed this paper again at the request of reviewer # and restructured the final paragraph of the paper (section 5).**

Table 1 is an excellent resource for future researchers.  It would help to note whether these plants are annual or perennial, and if the

**Added information on whether the plants are annual or perennial to the figure. The rest of this sentence was cut off, but I assume it follows the request in the top paragraph about the biology of elaiosome-bearing plants and timescales.**  
  
How frequently were ants sampled?

**Once in each plot for a timed trial. I added more information on the timing of the trial.**  
  
Line 191: This is not a complete sentence

**Edited. Line 191 now reads:**

***With respect to timing, this survey took place three-years of myrmecochore removal and a six-year waiting period.***  
  
207: I don’t think you need that comma

**Comma removed.**  
  
What composes Aphaenogaster diets?  Are they really elaisome specialists?  (that seems to be a necessary characteristic if this is indeed a specialist mutualism, as suggested at line 202)--\*\*\* actually, I just answered this question for myself by consulting your 2012 paper.  I suggest clarifying this in the intro, and perhaps elaborate on this ahead of your proposed hypothesis.  (why might you suggest ants are elaiosome-limited if they only facultatively feed on elaiosomes?)

**These are all good points, and it gets to the core question. I’d first state that a mutualism can be specialized without being obligate. I.e. there is clear evidence that *Aphaenogaster* are often the only species *effectively* dispersing seeds but the evidence that Aphaenogaster obligately depends on this food source is lacking. In other words, it is the plants that have become specialized to *Aphaenogaster*, perhaps not the other way around (but that would be an entirely new and interesting evolutionary question). However, lab experiments suggest that in controlled conditions elaiosomes can *provide* limiting nutrients to queens and larvae.**

**To get the reader on board with these points, I have updated the introduction and conclusion at multiple points. Both reviewers also made helpful suggestions along these lines.**

**For example, line 49 now reads:**

**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.**

**Line 68 (study system and predictions paragraph) now reads:**

**While Aphaenogaster are specialized as seed dispersing ants, it is less clear to what degree this is an obligate mutualism limiting their populations.**

219: Do you mean recruitment?  
**Change made to use the word “recruitment.”**

224: do you mean myrmechores?

**Change made to use plural “myrmecochores.”**  
  
228: I would strengthen this transition and delete “furthermore” because this sentence doesn’t compellingly build on the previous one.

**Good suggestion. I changed the sentence to follow better. Line 228 now reads:**

***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).***  
  
230: this sentence doesn’t obviously relate to previous ones either.  Are these all potential reasons that could explain why you didn’t see any impact on the ant community?  It’s not totally clear how each of these factors mentioned directly link with aphaenogaster activity.

**This original sentence did not fit in this location, so I moved it to the first paragraph of the discussion which focuses on how anthropogenic changes impacts seed-dispersal mutualisms.**

235: So it seems like you might need to have visited more than once annually to remove all the elaiosome resources?

**Possibly. However, I did not just remove seeds, but we also took to the laborious task of removing flower heads, recently fertilized flowers, and developing seed pods. The removal was times to occur when the plant species with the earliest phenology (Spring beauty) was ready to drop elaiosome-bearing seeds, and all other potential fruiting bodies (flowers, pods) were removed at this same time. Since all the plant species at Henry Buck site have an earlier flowering period, I am confident we removed the majority of elaiosomes that would be produced in a season.**

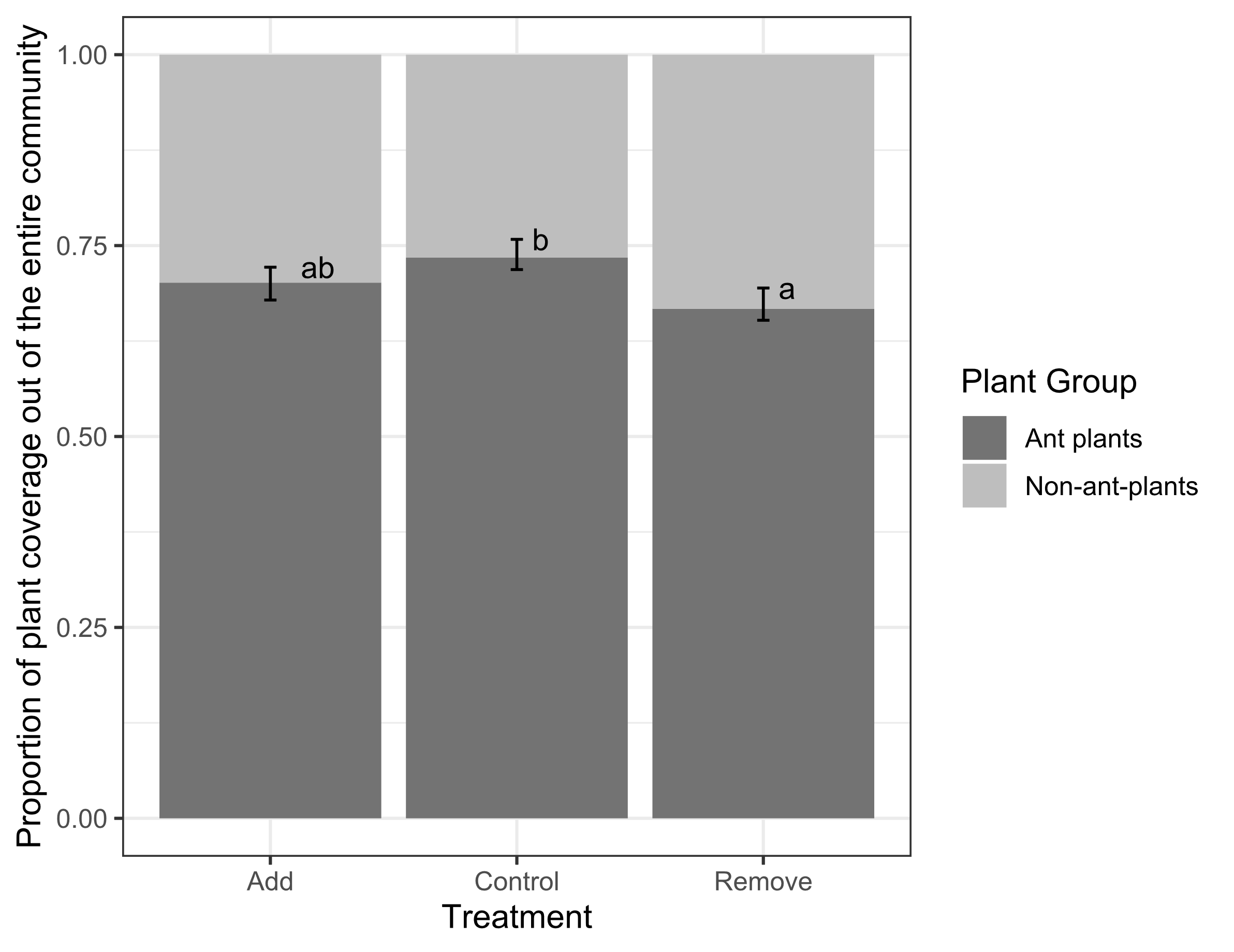
**To this point, I updated the methods to reflect that I did take this into account with the elaiosome removal effort.**  
  
254: This period should be a comma

**Line 254 was confusing as it was originally written, so instead I have rephrased these two final sentences in the paper to read:**

***In all, this study indicates that while elaiosomes represent a large pulse of nutrients for ants represented by a diversity of species found in the understory of Connecticut forests, this mutualism in resilient with respect to small-scale disturbance. Instead, further work should investigate how changes to climate or large-scale disturbances might negatively impact these seed-dispersal mutualisms in forest fragments.***  
Fig 1: Please add numbers of plants recorded in each category to your pie charts

**I did not count individual plants, but instead recorded cumulative coverage of plants in terms of linear basal area using a line transect. To address this comment, I added the centimeters of plant coverage in each of the 9 subplots to the figure. For example, in Figure 1, subplot 1 (control), there was 260cm coverage of spring beauty and 91cm coverage of trout lily. I have also updated the figure caption to reflect this. This change should also help to clarify the sampling methods for plant coverage, which was mentioned by both reviewers as a point that needed more clarity throughout the manuscript.**  
  
Fig 2. I would love to see the “after” composition of plants in each plot.  Could you turn this error plot into a stacked bar chart with the different species noted?

**Good suggestion, I agree that Fig 2. is sparse and can provide some more information not otherwise represented in Fig 1. and 3. However, I think it would be misleading to have a stacked chart with all the plant species since the analysis on the proportion of ant-dispersed plants vs. non-ant-dispersed plants and not a multivariate approach with all species. I also attempted a new plot but with stacks representing the proportion of each group and the axis starting at zero. However, due to the relatively small effect size of the treatment on proportions, it is difficult to visualize the differences among treatments at this 0-1 proportion scale. I have included the example here to verify that the editor/reviewers agree, but I am happy to substitute a figure at the request of the editor.**

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**If I understand the original comment, I believe the more important point is showing the “after” plant community composition among treatments. To this end, I have also modified the figure caption for Fig 1. This figure was already showing the “after” composition of the plant community among the nine sub-plots, providing some insight into the differences among three treatments. The previous figure caption was misleading since it stated Fig 1 shows the experimental design thus giving the impression it was the “before” composition.**

**Fig. 1 caption now reads:**

***Final plant community survey results and subplot layout for Henry Buck Trail plots. Each circle represents total plant coverage based on transects among nine 50m2 plots. Circle sections represent the proportional abundance of plants in each category: four common myrmecochores (Claytonia, Dicentra, Erythronium, Trillium), 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).***

**Fig. 2 remains unchanged, but editing this figure further is an option if the editors/reviewers deem it necessary.**  
  
Reviewer #1: This study posits two aims: to (1) survey the diversity of myrmecochores in Connecticut forests and (2) evaluate the elaiosome-limitation hypothesis at one site in said forests. Only the first aim is achieved, whereas several problems with the methodology used and lack of clarity in presentation of the methodology and results preclude this study from addressing the second aim. This study has potential and I would be excited to see these results published, but considerable reworking of the manuscript is needed to more thoroughly outline the methodology and/or reconsider certain approaches taken, clearly tie the methods used to the aims of the study, and more properly interpret the results considering the methods used. Specific comments on the writing are provided under "minor comments" while larger, manuscript-wide concerns are outlined in "major comments".

**These are very thorough suggestions, so I have made my best effort to address Reviewer 1s comments first and foremost. I accept that the test of the elaiosome-limitation hypothesis is narrow, so I have added significant text throughout, especially the discussion. In other words, the hypothesis is tested, but in one environment under a certain set of very specific conditions, and I do not want to give the impression this could be generalized to habitats were myrmecochores are rare or the plant species pool is biologically distinct. I hope the changes reflect this sentiment.**

**Among many other changes, I updated the hypothesis paragraph (last paragraph of the intro to read):**

***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.***

Minor comments  
L2: The comma following long-term feels unnecessary

**Agreed. To address other comments about the duration of the experiment, I also removed the mention of “long-term” in the title of the manuscript.**

**The title is: *Are seed-dispersing ants elaiosome-limited? An experimental test in a Connecticut forest dominated by myrmecochorous plants***

L66: Remove comma following King (or add commas elsewhere where a citation has two authors, e.g., L76. Either way, be consistent in citation style).

**Comma removed and elsewhere for sake of consistency.**

L101: Change "Clark and King et al. 2012" to "Clark and King (2012)"

**Change made.**

L102: Remove dash in "with-in"

**Dash removed.**

L108-116: What are the locations of the secondary forests surveyed? I see their coordinates are included in the captions for the supplementary figures, but a figure (even in the supplementary material) including their locations on a map may be useful. How were surveys conducted? Merely for presence/absence? How does L112-114 relate to quantifying richness as presented in Fig. S2-5? This section would benefit from greater detail of the methods used.

**I have opted to not include a map because the focus of the survey is not to ensure complete coverage of Connecticut’s ecoregions, but just to provide a baseline list of common myrmecochores. However, to the other point in this comment, methods were updated in the body of the manuscript and further details are provided in Appendix 1.**

**Lines 112 to 115 now read:**

***At each site, I designated transects starting at the habitat edge and continued inward to the center of the forest fragment. I measured the total coverage over our transect lines in cm (detailed transect methods provided in Appendix 1, Figs. S2-S5).***

L114-116: This is a prediction and thus would be better suited at the end of the introduction (e.g., after L84) rather than in the methods section.

**Change made. This section now follows the sentence originally in line 84.**

L118-144: Precision of language. "Removal of elaiosomes" insinuates you removed only the elaisosomes but left the seeds present at the site, rather than (what I am assuming you did) removing the diaspore, which would be the elaiosome and the seed together. Yes, the elaiosome is the important part here as that is the food source, but it is more precise to say you removed the diaspores, which include the elaiosomes.

**This section now states that *sources of elaiosomes* were removed. These include flower heads on myrmecochores, apparent flowers, developing seed pods, and any apparent diaspores.**

**For example, line 126 now reads:**

***By hand I removed the following: 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.***

L129: The ability to determine if supplementation of seeds can change Trillium coverage "long-term" is overstated here, and how this will be accomplished is not clear until the end of the results section. If this is going to be tested for, it needs to be a bigger part of the manuscript and more clearly incorporated into the introduction and methods sections.

**I agree that this is outside the scope of the stated hypothesis and methods. I removed any mention of testing impacts of seed supplementation on Trillium coverage, seed limitation, or dispersal effectiveness by ants. Instead, this is now described simply as a single supplementation treatment to test the elaiosome limitation hypothesis with a positive treatment rather than complete removal.**

**Line 130 now reads:**

***In a third positive treatment, I supplemented plots with all Trillium seed pods that were extracted from removal treatments. 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.***

L142-144: Provide more detail on how foraging activity was measured. See "major comments" section.

**To address this comment and others, I added an entire paragraph on the methods employed to report foraging activity of *Apheanogaster.***

**Line 146 now reads:**

***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 (Apheanogaster 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. One bait was placed in the center of each plot, while the other four baits were placed in the corner 2 meters from the plot border. After the 2-hour 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.***

L159-160: This feels like interpretation of the results (particularly the "rich assemblages" part), which should be relegated to the discussion.

**I removed sections here that sounded like interpretations of the results, and also reported information on the life history (perennial vs. annual) found in my plant database search.**

**Line 171 now reads:**

***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. With the exception of* Corydalis flavula *and* Melampyrum lineare*, all are perennial wildflowers.***

L170-171: No mention of surveys having occurred in spring 2017, or why they were conducted, have been clearly mentioned up to this point, so it is unclear how this result should be interpreted.

**I have addressed this comment by first editing the sentence originally at line 170-171 to specifically state that the source of these data were post-treatment plant community transects. I then added a short paragraph at the end of the field methods as this was the final piece of data collected, coinciding with the ant foraging activity survey.**

**Line 158 now reads:**

***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. Two line transects were laid diagonally in each plot, making a total transect length of 140m. This method gave detailed total coverage of every ant-dispersed plant species and all other plant species (pooled together as “non-ant-dispersed plants”).***

L189-198: Present means and standard deviations/errors of colony abundance.

**Significant edits were made to this entire paragraph, including new information on the mean and SE of the colony abundances.**

**Line 205 now reads:**

***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, χ2 = 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 ants to these baits (Kruskal-Wallis, χ2 = 0.78, df = 2, P = 0.67). For non-Apheanogaster 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.***

Major comments  
A clarification in the methodology is needed. Multiple times the study is referred to as "long-term", but the timeframe over which this study is conducted is unclear. In L139-142, the author states there is an "eight-year period (three-year removal, five-year sampling delay)" of the study, but then gives minimal details on when, where, or how the sampling of ant colonies were conducted.

Yes, cookie bates were used. But how many were set out? What was the baiting density? How long was foraging activity measured at each bait (or how was it even quantified)? Was the sampling of ant colonies conducted each year for five years after the cessation of elaiosome availability manipulation, or only at year 5? Some of the answers to these questions can be inferred from the results, but clarification is needed earlier on so that the reader can anticipate the results. Because of this lack of clarity, the use of the phrase "long-term" feels somewhat overstated as well, and only in the results section does the reader finally see that post-manipulation sampling occurred in 2017.

**To address this comment, I removed the term long-term from the manuscript body and title, and then added two paragraphs on the sampling methodology for quantifying ant colony activity.**

**I have added a supplemental figure (Fig S6) showing the layout of both cookie bait methods and line transect methods for the 2017 post-manipulation survey at the Henry Buck Trail site. Reference to this supplemental figure is also added to the text.**

Was Trillium the only species used to supplement elaiosomes as a food source (as appears to be the case in L126-127)? If so, that should be more clearly stated early on. This is a test of the "elaiosome-limitation hypothesis", but only using one species, not all myrmecochorous species present in the plot were used, incompletely testing this hypothesis. As such, it should be included as a caveat in the discussion, as the inclusion of elaiosomes from other species may lead to different results.

**Due to the timing of the experiment, only *Trillium* had all its diaspores simultaneously present to be moved in all three years of the study (otherwise flower heads, developing pods, etc. were present). I agree it is not ideal, but it is a small supplementation trail to test if a single species’ elaiosomes can impact ant activity level over multiple years. I have edited the hypotheses section, methods, and discussion to reflect this limitation.**

There is no true replication in this study as the design was only implemented at one site (Henry Buck Trail), so each treatment consists of 3 pseudoreplicates, not true replicates. The results must then be interpreted quite conservatively and not be broadly applied to this mutualism as it functions in deciduous forests across Connecticut or the northeast.

**This is addressed in the paragraph starting on line 239 of the discussion. I have added text to temper the conclusion and point out this is limited to a single forest fragment and not replicated across three sites.**

It is unclear how the statistical methods used match with the specific aims of the study. For example, why were GLMMs only used "for analysis of proportional plant coverage at Henry Buck" (L147-148) but not the other sites visited during the plant survey period? Was this analysis conducted on plant community data from 2009-10 or 2017? Or was it conducted to test for differences between pre- and post-manipulation? Again, some of this can be inferred from what is presented in the results, but it remains unclear which aim the author is addressing with this first analysis, so greater elaboration on exactly what is being tested for and which aim is being addressed with the GLMM is needed.

**The GLMM is to evaluate the treatment effects on plant community composition, while rarefaction is used to estimate species richness in the 2009-2010 surveys. I have edited the methods and results text to clarify this point in line 167.**

***To quantify the impact of treatments (i.e. 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).***

Further, the addition of evaluating the "treatment effects on… Trillium abundance" (L150-151) does not match either aim provided at the end of the introduction (survey Connecticut myrmecochores and assess the "elaiosome-limitation hypothesis"), nor has the narrative been set up adequately to prepare the reader for such an evaluation. This part of the analysis feels disjunct as the author has not described nor cited to sources describing how removing seeds for three years might affect plant populations, instead focusing on how doing so may affect ant populations.

**This section is now removed from the manuscript to focus entirely on ant populations as part of the primary hypothesis.**

I am not sold on the interpretation that "historical removal treatments significantly reduced the proportional coverage of all ant-dispersed plants compared to non-ant dispersed plants" (L174-175). In order to test this, I would have expected the following methodology: pre- and post-manipulation quantification of plant community coverage of myrmecochorous and non-myrmecochorous plant species in the sites where manipulation occurred (this was done by the author) followed by an analysis of the difference in community composition within each treatment from pre- to post-manipulation for each myrmecochorous and non-myrmecochorous groups (this was not completed).

**I do not have pre-treatment data from the 9 subplots, so this analysis cannot be done. Instead, the focus is on the response of the plant community to the manipulation treatment as measured in 2017, but the design would be more robust with longer-term data and prior knowledge of the plant community before the treatments were applied.**

**To address this limitation, I added the following text at line 252:**

**Finally, the experiment performed here could be improved with a longer-term removal and sampling regime in which the response to disturbance is measure with starting conditions of myrmecochore and *Aphaenogaster* populations.**

Figure 2 represents the "plot for binomial GLMM of % ant-dispersed plant cover out of all non-woody understory plants sampled" (L499-500), assumingly for the 2017 survey. However, if I am interpreting their results correctly, this only represents the post-manipulation proportion, and the Tukey's HSD is used to test for differences among treatments. All this indicates is a difference in the proportion of myrmecochores after manipulation, not a difference in the response of the proportion of myrmecochores to the manipulation, thus failing to address the mechanism. For example, this post-manipulation difference could be unrelated to the treatment, simply reflecting 'random' differences in the distribution of myrmecochores across treatments within the site. It is unclear whether this is an actual reduction in myrmecochore cover following the seed removal treatment (supporting the author's statement), or whether there is already lower cover in these plots for reasons unrelated (not supporting their statement). Thus, their statement "these results suggest that the manipulation only moderately reduced the proportional abundance of myrmecochores" (L185-187) is unfounded. Further, there is no simultaneous test for whether the same trend holds true for non-myrmecochores which would be necessary to assess whether the drop in abundance of myrmecochores was a result of removing seeds or a result of plant community-wide factors (e.g., drought, browsing, etc.).

**I agree that a before-and-after comparison would be a much more powerful approach. However, we used a structured block design in which plots were assigned treatments with no prior knowledge of the site conditions. In the GLMM hypothesis testing approach, the p-values being calculated are estimating whether the differences in proportional abundance of myrmecochores are likely due to random chance or not. I say this because it is always possible any community-wide factors could have happened in these sites over the survey period, from drought, browsing, etc. However, the probability that these impacts occurred in onlythe three treatment plots and notin the controls is very low. Knowing prior conditions could rule that out definitively, but I do not have those data.**

It appears the NMDS was used to test for this latter point, but no visualization is provided for the reader to validate the author's interpretation, nor is it clear how the use of the GLMM and NMDS relate.

**Rather than add another figure for the NMDS, I felt it was better to leave this analysis out. In the revisions of the manuscript, it became clear it did not fit. The focus of the experiment is on ants and myrmecochore abundance rather than specific impacts to plant community composition. There were no mentions of this analysis in the introduction or conclusion in the original version of the manuscript anyways. I removed mention of NMDS from one sentence in the methods. Species richness estimates using the VEGAN package in R (supplemental figures) are retained, though.**

**In the figure caption for Fig. 1., I removed:**

***There was no significant difference in plant community composition across the three treatments (NMDS, Permutation test for treatments, r2 = 0.0218, P = 0.329).***

**From the results section I removed*:***

***At the community-wide level, I observed no significant change in the composition of the plant community when applying NMDS across all herbaceous plant species (NMDS, Permutation test for treatments, r2 = 0.0218, P = 0.329).***

The same concern of assessing plant community responses to the treatments holds for the ant community, as no pre-manipulation colony abundance measurements were made against which post-manipulation colony abundance measurements can be compared to. It appears the Krustal-Wallace test was only used to test for differences among colony abundances post-manipulation, so differences in post-manipulation abundance among treatments cannot be directly tied to the effect of the treatments themselves. There is some ability for the author to posit that removing elaiosomes does not cause reduced colony abundance because there was no difference in ant visitation to baits among treatments, but a key component to that interpretation is missing: actually quantifying changes (potential increases or decreases) in colony abundance/visitation. This, plus the fact that this study was not replicated at multiple sites, necessitates these results be interpreted with abundant caution.  
  
**I agree that pre- and post-manipulation surveys would greatly strengthen the experiment, but pre-treatment data is not available. Instead, I am relying on the random systematic sampling design of the experiment. Removal plots were chosen without any knowledge of the ant or plant community, so there should not be any bias in those offsetting the impacts of the treatments.**

**At the core, my predictions are quite narrow – removing sources of elaiosomes should reduce ant populations relative to a control. I cannot broader the predictions given the limitations of this design, which I acknowledge. With that in mind I have added an additional section to the discussion highlighting this short-coming, with additional information provided on the biology of *Aphaenogaster* relevant to this limitation.**

**Line 234 to 243 now reads:**

***Likewise, 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 *abundance (Warren et al. 2019). However, my results should be interpreted carefully as the study area only encompasses a single population of* Aphaenogaster *colonies. These 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* Apheanogaster *collected from Connecticut forests show elaiosomes could be an important source of nutrients for brood production by queens (Clark and King, 2012).***

Reviewer #2: Are seed-dispersing ants elaiosome-limited? A long-term, experimental test in a Connecticut forest dominated by myrmecochorous plants  
  
Summary of paper and general recommendation:  
The manuscript "Are seed-dispersing ant elaiosome-limited? A long-term, experimental test in a Connecticut forest dominated by myrmecochorous plants" by R. Clark has two main goals. First, to describe the native myrmecochore plant community within three Connecticut forests and second, to test the "elaiosome-limitation hypothesis".  The study combines literature searches and field surveys to create lists of herbaceous, myrmecochore plants found specifically in Connecticut deciduous forests. In order to test the "elaiosome-limitation hypothesis" they conducted a manipulative experiment in which they removed or added of elaiosome food sources, including controls, and measured myrmecochore plant percent cover and the abundance of ant colonies. 25 species of myrmecochorous species were identified in the forest sites, demonstrating a rich community assemblage within these forests. In addition, they found myrmecochore plant cover significantly lower in seed removal plots compared  
to controls, but myrmecochore plant cover remained the dominant cover type. Myrmecochore plant cover did not increase in seed addition plots, Finally, elaiosome removal and addition treatments did not have a significant effect on mutualist ant (Aphaenogaster sp.) colony abundances. Overall, the study seeks to answer whether seed-dispersing ant colony abundance is sensitive to elaiosome food resource removal and disturbance, and whether this mutualism is at risk as forests increasingly undergo anthropogenic change.

Overall, it was a true pleasure to read this manuscript and I think it makes an exciting contribution to understanding the dynamics of the specialized mutualism of ant-mediated seed dispersal. The manuscript supports an increasing body of work that suggests that while elaiosome food resources provide energy and nutrient benefits to ant colonies, colony abundance is not limited by elaiosome availability. In addition, the undertaking of removing > 155,000 flowers or seed pods is an impressive feat! My primary critiques include the need for more detail within the methodology regarding the ant colony surveying and vegetation surveys and reframing of results to represent the treatments being tested more accurately. I also suggest adding to the discussion section, the consideration of plant growth form and age in the context of plant cover responses. I have detailed these major suggestions below, as well as some more specific comments.

**I thank the reviewer for their supportive comments. To address the major points here, I have added more information on the methodology to the manuscript, especially the use of cookie baits to measure colony activity and the way plant coverage was quantified. I also added a paragraph to the discussion about plant growth form and life history, especially given the new revisions reveal that the 23 out of 25 of ant-dispersed plants in this project were perennial species.**

**Among several other changes, line 228 now reads.**

***This ability of myrmecochories 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.***  
  
Broad suggestions:  
The first major suggestion is additional detail regarding quantifying myrmecochore percent cover data. Survey transects are mentioned in the Methods section however I think including the number of transect replicates and location within the plots would help with clarity. Similarly, additional detail for the ant colony surveys is needed. Ant baits are mentioned; however, the number and location of these baits are not included, and I think including this information would provide necessary context of how data was collected. For example, if baits were located along edges of plots, would there be influence of migration of species from adjacent areas?

**These are good suggestions, and this information has been added at the request of the editor and the first reviewer.**

Another major suggestion is some discussion of the ephemeral nature of Aphaenogaster colonies (Lubertazzi 2012) and how this colony trait and general colony migration could impact the results seen in the experiment. We know that Aphaenogaster colonies move nest locations seasonally due to productivity shifts between the summer and winter.

**This is a good point and a common challenge when tracking *Aphaenogaster* populations. I thought the best course of action was the mention this in the discussion when highlighting the response of the ant community (or lack-there-of) to the removal of sources of elaiosomes.**

**Line 247 is now added:**

**Aphaenogaster rudis *group ants form ephemeral nests in spring and summer, moving to more permanent locations to overwinter (Lubbertazzi 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.***

An additional suggestion is to include some discussion of plant growth form and plant age in the interpretation of myrmecochore plant cover to elaiosome removal treatments. The author clearly describes the median age of ant colonies compared to the length of the experiment duration. Seeing as this is a mutualism, I think including discussion of myrmecochore plant ecology would compliment the interpretation of the results. For example, since Trillium erectum is a perennial, do we expect population changes after seed removal for 8 years? In addition, do other myrmecochore species reproduce from rhizomes? I find this information would help clarify some of the conclusions in the manuscript.

**I have added information on this point at several points of the manuscript at the request of reviewer 1 and the editor as well. However, to this specific point from reviwer 2, I added the following stipulation to the conclusion paragraph on our sampling regime caveats.**

**Line 266 now reads:**

***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, one of the major conclusions from the manuscript is that Trillium seed addition resulted in intermediate myrmecochore cover compared to removal and control plots. However, the comparison of total myrmecochore cover as a response of Trillium seed addition seems inappropriate since total myrmecochore cover would not change due to the addition of a single plant species. I suggest reframing the results and to compare Trillium plant responses to Trillium seed addition and removal.

**I agree the comparison to removal and supplementation is important. Rather than remove the current analysis, I modified the analysis to include a comparison of both. A second sentence on the planned comparison of supplementation and removal has been added at line 200.**

***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).***

**The methods have been updated to address this point and other comments regarding more description of the Trillium analyses.**

**Line 172 now reads:**

***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.***  
  
Specific comments:  
Line 27-29: This sentence is a bit awkward.

***Consequently, evidence that Aphaenogaster were limited by elaiosomes was limited, supporting more recent research indicating that ant population densities are not constrained by the availability of myrmecochores.***

**Changed to:**

***Our results provide some evidence that in habitats where myrmecochores dominate, Aphaenogaster populations may not always be limited by the availability of elaiosomes.***

Line 59-62: This section conflicts with arguments made in the conclusion, regarding the  specialization of Aphaenogaster ants as "keystone" dispersers. It is not clear whether this work supports or challenges this claim, and I wonder if the Introduction and Conclusion could be modified to clarify.

**In eastern 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 (Smith et al. 1989), which prevents seed predation (Heithaus 1981, Ruhren and Dudash 1996).**

**I’ve removed the mention of keystone since it does indeed undercut the introduction. This text in line 288 has been changed to:**

**Aphaenogaster rudis *are often the most effective ant species with regard to successful seed dispersal of many myrmecochore species (Ness et al. 2009). Despite this specialization from the perspective of the plant, more recent evidence suggests* Aphaenogaster *are dependent on elaiosomes only under certain contexts (Clark and King 2012, Warren et al. 2019).***

Line 85-87: It is mentioned that the elaiosome removal experiment is within the "context of this mutualistic community to tolerate disturbance". It could be helpful within the methods section to specifically define the use of the term "disturbance" within the context of this paper. I only bring this up since the author mentions examples of forest "disturbance" (fragmentation, clearing) in the intro which can be confusing when using "disturbance" for ant food resource removal.

The original line (below) was updated to reflect suggested changes to the hypotheses and results:

**Moreover, there are relatively few studies indicating the importance of elaiosomes for maintaining populations of *Aphaenogaster* over multiple years, particularly in the context of the ability of this mutualistic community to tolerate disturbance.**

**Because this hypothesis is not testing ecological disturbance specifically. This line has been changed to:**

**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.**

Line 93: myrmecochore is missing an "e" at the end

**This is intentional. A query specified this way will provide all matches that contain the string “myrmecochor”, including myrmecochorous, myrmecochory, and myrmecochore.**

Line 98-104: These sentences are somewhat hard to follow. If I understand correctly, literature searches were conducted to gather a list of myrmecochorous species found in Connecticut, but only species with records of observed removal were included. Was this process then repeated for any novel plant species found in the field surveys?

**I have rewritten this section and it has been changed to:**

***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 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.***

Line 117: What is meant by a "disturbance trail"?

**That is a typo. Given prior comments about using disturbance instead of “elaiosome removal” I have changed the sub-header to simply read: *Simulating loss of elaiosomes at Henry Buck Trail***

Line 121-123: Monitoring and removing myrmecochore seed pods is a great challenge due to how quickly fruits can form and dehiscence. Some addition clarification here would be helpful, seeing as plots were visited once a month suggesting the possibility that some elaiosomes were missed between visits. Is this the justification for why flowers were also collected before fruits formed? I think including justification of flower removal would clarify this.

**This text has been updated in revisions addressing a previous comment. The quoted text now reads:**

**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.**

Line 125-126: Did the Trillium seeds need to be preserved and where seeds used from the same year that they were harvested? I just wanted to make sure seeing as freezing could have an impact on seed viability. Additionally, I would find it helpful to include the justification behind picking Trillium erectum.

**These were moved immediately as I was able to time the treatment just prior to seed release by *Trillium erectum.***

**To address this comment, I mentioned that this transfer happened immediately and that seeds were not brought back to a lab or freezer.**

**Line 138 now reads:**

**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.**

Line 129-132: This sentence is hard to follow. What is the "indirect" test referring to?

**This sentence has been removed in editing to tighten up the *Trillium* section of the manuscript.**

Line 138: "our" with "or"

**This sentence has been removed in editing following comments from reviewer #1.**

Line 147-149: What were the fixed and random effects for the GLMMs?

**This information has been added. Line 174 now reads:**

***All GLMMs used block as a random effect and elaiosome manipulation treatment as a fixed effect.***

Line 165-166: It is mentioned that "the highest % cover of ant-dispersed plants was observed at Henry Buck Trail" and refers to Figure S2, however this figure illustrates plant richness.

**It was true that Henry Buck had the highest % cover, it is indeed more clear to state Figure S2 describes species richness.**

**Line 192 has been changed to:**

**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).**

Line 174-175: This sentence is hard to follow. Is the comparison being made between different treatment types or the amount of myrmecochore vs. non-myrmecochore plants?

**The original sentence (below) was not worded clearly. My hand manipulation reduced proportion of myrmecochores out of the total herbaceous plant community. The effect size is small but still significant. The %’s from these proportions are shown in lines 206-207.**

***Our historical removal treatments significantly reduced the proportional coverage of all ant-dispersed plants compared to non-ant-dispersed plants (Fig 2, Binomial GLMM, χ2 = 16.85, df = 2, P < 0.001).***

**Has been changed to:**

***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, χ2 = 16.85, df = 2, P < 0.001).***

Line 179-181: How were Trillium plants counts quantified? Was this part of the transect surveys? It would be helpful to clarify here or in the Methods section.

**Both sections and model information have been updated to reflect what is being measured in the Trillium counts regarding prior comments. Trillium is being modeled as the number of cm of coverage in the updated analysis.**

Line 196-197: The author mentions "recruitment of non-Aphaenogaster ants" and refers to Fig. 3. However, Fig. 3 shows Aphaenogaster colony abundances.

**I have specified that this is recruitment of non-*Aphaenogaster* ant colonies.**

Line 214: italicize Aphaenogaster

**Change made.**

Line 223-226: This is great insight into what you might be seeing, and I agree!

**Thank you. It is promising that the loss of a few generations of elaiosomes do not devastate mutualistic partners, but it should be tested further in environments where myrmecochores are less dominant.**

Table 1: I find that the table description and table contents do not match here. For example, the author mentions that Dicentra canadensis and Trillium cernuum do not have empirical evidence to suggest ant recruitment of these seeds. However, they are included in the table. Clarification would be helpful here. The updated nomenclature for Hepatica acutiloba is Anemone acutiloba according to GoBotany.com.

**To the first point I have added text to the methods describing the rationale for including these species. All their close relatives (genus or species complex) are ant-dispersed, and they have a similar life history to their ant-dispersed kin. Several studies also include them as ant-dispersed plants, however the behavioral assay criteria was not met. I suspected it would be misleading to exclude these two species from a list of Connecticut myrmecochores as they are common forest plants.**

**To the second point, I agree the many nomenclature changes for *Hepatica* and *Anenome* is confusing, and this is the result of many systematic revisions in the last 30 years. To be consistent, I have gone with the naming scheme from the original publication and then provided text on the date names for the floristic region (New England). Without having a specimen present from the 1981 surveys and 2014 experiments, it is unclear which member of the revised genus was originally described. I have also referred to a colleague who is an expert on spring ephemeral ecology (Dr. Jack Tessier, Professor and Plant Ecologist at State University of New York at Delhi) to verify the names on my species list. An acknowledgement has been added to the manuscript.**

Fig. 1: Are all the members of the listed genera (Claytonia, Dicentra, Trillium) combined? The addition of numbers for each response category could help reader interpretation.

Numbers have been added at the suggestion of the handling editor. At Henry Buck, only one species of Claytonia, Dicentra, and Trillium were found within each of these genera. In other words, no species were combined within these groups – each category represents one species other than the “other myrmecochores” pie slice.

**To clarify, line 539 now reads:**

***Circle sections represent the proportional abundance of plants in each category: four common myrmecochore species (Claytonia virginica, Dicentra cucularria, Erythronium americanum, Trillium erectum), other myrmecochores, and all non-myrmecochore herbaceous plants.***

Fig. 2: Very clear and neat figure! Well done!

**Cheers!**

Fig. 3: A "recruitment line" is mentioned here but not defined anywhere in the manuscript.

**I agree this can be clarified. Line 549 now reads:**

***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.***