

Functional resilience to disturbance

Historical forest disturbance results in variation in functional resilience of seed dispersal mutualisms

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1 Abstract

2 Mutualistic interactions provide essential ecosystem functions, such as promoting and
3 maintaining diversity. Understanding if functionally important mutualisms are resilient (able to
4 resist and recover) to anthropogenic disturbance is important to understand the capacity for
5 diversity to recover. Animal-mediated seed dispersal supports plant population growth and
6 community structure, and disturbance of this function can threaten plant diversity and contribute
7 to low resiliency. Ant-mediated seed dispersal mutualisms are particularly sensitive to
8 anthropogenic disturbance, as they rely on one to a few high-quality dispersal partners. In North
9 American eastern deciduous forests (NAEDF), ants in the genus *Aphaenogaster* are “keystone
10 dispersers” of 30-40% of understory forbs adapted to dispersal by ants (myrmecochores). The
11 majority of present day NAEDF have regenerated from previous disturbance in the form of
12 historical land use change (HLUC), due to clearing for agriculture. Previous studies have
13 revealed that myrmecochore diversity is not resilient to HLUC. Here, we ask if seed dispersal
14 mutualisms are resilient to HLUC and if decreases in mutualistic interactions with partners,
15 *Aphaenogaster* sp., or increases in antagonistic interactions cause degradation of function. In a
16 large-scale natural experiment (20 sites), we measured seed removal, the abundance of
17 mutualistic partners and other invertebrates interacting with seeds, myrmecochore cover and
18 diversity, along with ant habitat and forest structure. We found lower and more variable seed
19 removal in secondary forests compared to remnant forests. A path analysis of all forests revealed
20 that abundance of mutualists was the primary determinant of variation in seed removal, and that
21 seed damage by antagonists (invasive slugs) negatively affected dispersal and was higher in
22 secondary forests. In a path analysis of remnant forests, the link between mutualist abundance
23 and seed removal was absent, but present in the secondary forest path, suggesting that seed
24 dispersal is more variable and dependent on mutualist abundance in secondary forests and is
25 stable and high in remnant forests. Here we show that functional resilience to HLUC is variable
26 and may impede recovery of understory plant communities. This work provides key insights on
27 the effects of anthropogenic disturbance on mutualistic interactions and how the resilience of
28 critical ecosystem functions impacts diversity resiliency.

29
30 **Keywords:** land use change, seed dispersal, mutualism, myrmecochory, resilience, ecosystem
31 function, *Aphaenogaster*, *Arion*, ants, animal–plant interaction, deciduous forests

32 Introduction

34 Mutualistic interactions provide essential ecosystem functions and contribute to the
35 maintenance of diversity in ecosystems (Goheen & Palmer 2010; Prior et al. 2015; Kaiser-
36 Bunbury et al. 2017; Wandrag et al. 2017). Understanding if mutualistic interactions are

37 functionally resilient (able to resist and recover) to anthropogenic disturbances will reveal if
38 diversity benefitting from interactions has the capacity for recovery (Oliver et al. 2015; Kaiser-
39 Bunbury et al. 2017; Rogers et al. 2017). Shifts in anthropogenic land use leave ecosystems to
40 naturally regenerate from wide-scale disturbance, and variation in legacies may prevent or alter
41 recovery trajectories, leading to reduced or altered interactions and diversity (Holling 1973;
42 Suding et al. 2004; Sabatini et al. 2014; Kaiser-Bunbury et al. 2017). Whether or not functionally
43 important mutualistic interactions are resilient to anthropogenic disturbance is an open question
44 necessary to uncover the capacity for recovery of diversity in ecosystems.

45 Animal-mediated seed dispersal is a functionally important mutualism (Schupp 1993;
46 Ness et al. 2009; Rogers et al. 2017; Wandrag et al. 2017), where plants benefit from having a
47 dispersal agent that increases dispersal distance from maternal plants, protection from seed
48 predators, and directed dispersal to more favorable location (Tiffney & Mazer 1995; Kalisz et al.
49 1999; Wenny 2001; Bronstein et al. 2006; Giladi 2006). Animal-mediated seed dispersal benefits
50 restoration, given its positive role in plant population growth and distribution (Wunderle 1997;
51 da Silva et al. 2015; De Almeida et al. 2020), and uncovering if seed dispersal is resilient post
52 disturbance can better inform restoration planning.

53 Myrmecochory, or ant-mediated seed dispersal, is a widespread dispersal syndrome,
54 including ~11,000 plant species worldwide (Lengyel et al. 2009). Myrmecochorous plants are
55 adapted to dispersal by ants, having a lipid-rich appendage (elaiosome) that attracts ants and
56 provides a food reward. Myrmecochory is a particularly specialized diffuse mutualism, with one
57 or a handful of ant species dispersing most seeds (Bronstein et al. 2006; Giladi 2006; Gove et al.
58 2007; Manzaneda & Rey 2009; Ness et al. 2009; Warren et al. 2014). Asymmetrical diffuse
59 mutualisms are sensitive to anthropogenic disturbance, as changes in the presence, abundance,
60 and interaction of effective animal disperser partners can disrupt interactions (Schupp 1993;
61 Traveset & Richardson 2006; Schleuning et al. 2011; Prior et al. 2015), with cascading impacts
62 on plant communities (Christian 2001; Prior et al. 2015; Rogers et al. 2017).

63 North American eastern deciduous forests (NAEDF) are a myrmecochory hotspot
64 (Lengyel et al. 2009) with 30-40% of understory forbs possessing elaiosomes (Beattie & Culver
65 1981; Handel et al. 1981). High-quality dispersers find seeds quickly, do not harm seeds, and
66 deposit seeds outside of nests (Giladi 2006; Canner et al. 2012; Prior et al. 2014; Prior et al.
67 2015; Gordon et al. 2019; Meadley Dunphy et al. 2020). Poor-quality dispersers interact with
68 seeds but fail to disperse seeds and might damage seeds (Christian 2001; Bronstein et al. 2006;
69 Giladi 2006; Stuble et al. 2011; Warren & Giladi 2014; Parker et al. 2021). In NAEDF, ants
70 belonging to the genus *Aphaenogaster* are considered "keystone" dispersers, responsible for
71 majority of dispersal events (Ness et al. 2009).

72 NAEDF are impacted by various types of anthropogenic disturbances (Reich & Frelich
73 2002). Most contemporary forests are secondary, having passively regenerated from historical
74 clearing for agriculture or timber harvesting (Flinn & Vellend 2005; Flinn & Marks 2007).
75 Herbaceous understory communities are not resilient to historical clearing with secondary forests
76 lacking diversity, especially of myrmecochores, (Bellemare et al. 2002; Mitchell et al. 2002;
77 Flinn & Vellend 2005; Griffiths & McGee 2018). Several factors likely contribute to low
78 resiliency, including recruitment limitation to secondary fragments from source populations
79 (Flinn & Vellend 2005). Agricultural disturbances leave legacy effects in soils that impact
80 herbaceous plants such as elevated pH and nutrients and lower organic matter (Koerner et al.
81 1997; Dyer 2010). Less is known about whether functionally important interactions, such as seed

82 dispersal, are resilient to historical land use change (HLUC) and if reduced function contributes
83 to low understory recovery (except see Mitchell et al. 2002; Kiel et al. 2020; Parker et al. 2021).

84 Reduction in ant partner presence, abundance, or interactions could slow understory
85 recovery in secondary forests (Mitchell et al. 2002; Kiel et al. 2020; Parker et al. 2021). It is well
86 established that *Aphaenogaster* presence and abundance positively influences seed dispersal,
87 distribution and community structure of myrmecochores (Kalisz et al. 1999; Ness et al. 2009;
88 Warren & Giladi 2014; Prior et al. 2015). One way in which myrmecochory may not be resilient
89 to HLUC is if the keystone mutualist, *Aphaenogaster*, are absent or have lower abundances.
90 Prior clearing or soil disturbance could reduce *Aphaenogaster* abundance, and recovery from
91 populations in intact forests could be limited (Schmidt et al. 2013). Ant abundance in secondary
92 forests could also be negatively impacted by changes to forest floor conditions, including altered
93 canopy structure influencing soil microhabitat (Warren et al. 2012), or altered leaf litter and
94 woody debris, which provides ant nesting habitat (Lubertazzi 2012).

95 Myrmecochory might also not be resilient to HLUC if there is an increase in organisms
96 that interact antagonistically with *Aphaenogaster* or seeds. *Aphaenogaster* is generally
97 subordinate to other ant species and competition with other ants could reduce *Aphaenogaster*
98 abundance (Ness 2004; Warren et al. 2020; Parker et al. 2021). Other ants in NAEDF are lower
99 quality dispersers that fail to disperse seeds or harm seeds (Ness et al. 2004; Giladi 2006;
100 Rodriguez-Cabal et al. 2012; Parker et al. 2021). If secondary forest conditions favor other ant
101 species, they could disrupt seed dispersal directly by harming seeds, failing to disperse seeds,
102 moving them short distances, or indirectly by competing with *Aphaenogaster* and reducing their
103 effectiveness (Ness 2004; Leal et al. 2015; Parker et al. 2021). Finally, other invertebrate
104 organisms interact with myrmecochore seeds and could affect dispersal (Gunther & Lanza 1989;
105 Jules 1998). For example, in NAEDF, the invasive slug, *Arion subfuscus*, occurs in disturbed
106 forest habitats, such as forest edges, and damage myrmecochorous seeds by robbing elaiosomes
107 and preventing dispersal (Meadley Dunphy et al. 2016; Kiel et al. 2020; Parker et al. 2021).

108 Here, we examine if seed dispersal mutualisms are resilient to wide-scale disturbances
109 resulting from historical land use change (HLUC). In particular, we ask whether seed dispersal is
110 lower in secondary forests compared to remnant forests, and if so, whether decreases in
111 interactions with mutualistic partners or increases in antagonistic interactions contributes to
112 degradation of function. We conducted a natural experiment in 20 paired remnant and secondary
113 forests, where we compared seed dispersal of myrmecochorous seeds, along with myrmecochore
114 cover and diversity. To uncover how abundances of organisms interacting with seeds affects
115 variation in dispersal, we measured the abundance of the keystone disperser, along with other
116 invertebrates (ants, slugs) interacting with seeds. We also measured several forest structure and
117 ant habitat factors (canopy cover, ant nesting sites, and abiotic factors) and performed a path
118 analysis to reveal how forest conditions affect the abundance of mutualistic and antagonistic
119 organisms interacting with seeds and seed dispersal. We predict that seed dispersal will not be
120 resilient to HLUC and be lower in secondary forests due to lower abundance or fewer
121 interactions with mutualists or increased abundance or interactions with antagonists. We also
122 predict a disruption in seed dispersal mutualisms may relate to lower myrmecochore diversity
123 and cover in secondary forests. Better understanding the effects of anthropogenic disturbance on
124 this critical mutualistic interaction, in particular its effect on mutualists and antagonists, provides
125 key insights into the functional resiliency of mutualisms, and its influences on the resiliency of
126 diversity. Understanding resiliency of seed dispersal mutualisms has important implications for
127 restoring understory herbaceous plant communities.

128 **Materials and Methods**

129 Study sites

130 We conducted this study across 20 paired sites in mesic hardwood forests in NAEDF.
131 Each pair of sites included a remnant forest site (no history of clear cutting for agriculture ≥ 150
132 years) and a secondary site (regenerated from agricultural use (clear cut for pasture or plowing)
133 50-75 years). Remnant sites were located and verified using historic maps, aerial photographs,
134 literature references, management reports, and land manager interviews (see details in Appendix
135 S1). To mirror topographic conditions in the remnant sites, we selected secondary sites adjacent
136 to or geographically close (within 32 km) to remnant sites. We grouped sites into three
137 ecoregions (E1-E3) to account for regional variation (Appendix S1: Fig. S1).

138 At each study site, we set up three 50 m survey transects away from forest edges (> 100
139 m). Transects averaged 80 m from each other and were placed in areas with at least $\sim 75\%$
140 deciduous tree cover. We created 5 m² survey plots that alternated along transects with different
141 plot types: seed removal, invertebrate community, and abiotic characteristic plots; vegetation
142 plots; and ant habitat plots (Appendix S1: Fig. S2).

143

144 Study system and species

145 *Aphaenogaster* are part of the *Aphaenogaster-rudis-fulva-texanus* species complex.
146 (Umphrey 1996; Ellison et al. 2012; DeMarco & Cognato 2016). In our sites, we found *A. rudis*
147 and *A. picea* (that are polyphyletic and challenging to delineate morphologically) and
148 monophyletic *A. fulva* (Parker et al. 2021; Buono et al. 2022; Quartuccia & Buono, *unpublished*
149 *data*) and refer to this group as *Aphaenogaster* sp. in our study. *Aphaenogaster* are abundant ants
150 in NAEDF, with up to 2 nests per m². They form single queen colonies (monogynous) with
151 several hundred workers (Lubertazzi 2012). They are the primary dispersers of myrmecochores
152 in NAEDF (Beattie & Culver 1981; Handel et al. 1981), including many species that we found at
153 our sites, including *Trillium* sp., *Sanguinaria canadensis*, *Anemone acutiloba*, and *Asarum*
154 *canadense* (Appendix S1: Table S3).

155 Other invertebrate organisms that are known to interact with myrmecochorous seeds in
156 deciduous forests include other ant species (e.g., *Lasius americanus*, *Myrmica punctiventris*, and
157 *Camponotus pennsylvanicus*) that are low-quality dispersers (Giladi 2006; Ness et al. 2009;
158 Warren et al. 2015; Parker et al. 2021) (Appendix S1: Table S4). We also commonly observed
159 the invasive slug belonging to the *Arion-subfuscus-fuscus* species complex interacting with
160 seeds. This species complex is native to Europe and though both putative species are observed in
161 North America, we refer to this slug as *A. subfuscus*, seeing as *A. subfuscus* is the more wide-
162 ranging species (Pinceel et al. 2004). The slugs can be found in various habitats (forests, fields)
163 and is a described pest, feeding on plants and fungi (Beyer & Saari 1978).

164

165 Seed dispersal and invertebrate organisms interacting with seeds

166 We performed seed removal trials between late June to mid July 2018-2019 on non-rainy
167 days to test if seed removal by ants differed between remnant and secondary sites. In each site,
168 we set up 15 seed depots in seed removal/invertebrate community plots along three transects
169 (Appendix S1: Fig. S2). Depots were located in the center of plots and consisted of a 10 cm³ petri
170 dish placed on top of a paper index card (10x15 cm). Seeds from *A. canadense*, were collected
171 when fruits were dehiscing from local sites. We chose *A. canadense*, as it is a common
172 myrmecochore in this region (although not endemic to all sites; Appendix S1: Table S1) and is
173 preferred by *Aphaenogaster* (Prior et al. 2015; Buono et al. 2022). To avoid unintentional

174 introduction, seeds were frozen at -80 °C for 24 hr. (to render seeds inviable) and then stored at
175 21 °C. Seeds were thawed before placing them on depots and freezing is not known to impact ant
176 interactions with seeds (Zelikova et al. 2008). On the day of the trials, we placed 8 seeds on
177 depots. Wire mesh cages (7x12x12 cm) with 1.3 cm² holes were secured over depots to exclude
178 rodents but allow for invertebrate access. Depots were set out in the late morning (~11:00 am) on
179 non-rainy days. We observed depots 1/hr. for 3 hrs. During each observation, we recorded the
180 number of seeds remaining, the presence and species of ants in depots, the type of interaction
181 (carrying, handling), and the identification of any other invertebrates (i.e., slugs). Final
182 observations were taken after 24 hrs., where we recorded the condition of the elaiosome (intact,
183 partially removed, or completely scooped out), and the identification of any organisms on the
184 depot (Meadley Dunphy et al. 2016; Parker et al. 2021).

185 We set out pitfall traps during the seed removal trials to compare *Aphaenogaster* sp.
186 abundance, other ant abundance and diversity, and slug abundance between remnant and
187 secondary sites. At each site we set up 30 pitfall traps (2 in each seed removal plot (Appendix
188 S1: Fig S2)). The pitfall traps included a plastic cup (9 fluid oz., 7 cm tall, 9 cm diameter) filled
189 with ~ 3.5 oz of soapy water (with biodegradable soap) and depressed into the soil, top level with
190 the ground. A 5 cm² wire mesh grid with 1.3 cm² openings covered the opening of the cups.
191 Pitfall trap surveys were conducted on non-rainy days and left out for 24 hrs. Contents of traps
192 were preserved in 70% ethanol for identification. We pinned specimens of ant species and
193 identified all ants to the lowest taxonomic unit using regional keys (Ellison et al. 2012).

194

195 *Myrmecochore diversity and vegetation structure*

196 We conducted vegetation surveys to compare differences in myrmecochore diversity and
197 vegetation structure between remnant and secondary sites. Surveys were designed to compare
198 forest composition and cover at several levels (understory, shrub, tree, and canopy) (Davison &
199 Forman 1982). We set up four 1 m² quadrats in the corners of each vegetative plot to measure
200 herbaceous cover. We categorized plant species with seeds known to bear elaiosomes as
201 myrmecochores and identified each to the lowest taxonomic unit (Handel et al. 1981; Bellemare
202 et al. 2002) (Appendix S1: Table S3). In 5 m² vegetative plots, we also measured shrub cover,
203 tree basal area, percent canopy openness and identified tree species (see details in Appendix S1).

204

205 *Abiotic characteristics and potential ant habitat*

206 In seed removal plots, we measured air temperature and three soil characteristics: soil pH,
207 moisture, and temperature during the seed removal trials (see details in Appendix S1). We
208 surveyed three known ant habitat types (leaf litter, decaying logs, and movable rocks (i.e., not
209 large boulders)) in three plots (Appendix S1: Fig. S2) (see details in Appendix S1). For each
210 habitat type, we recorded the presence of ant colonies (Appendix S1: Fig. S4).

211

212 *Statistical analysis*

213 We performed binomial generalized linear mixed effects models (GLMM) on the number
214 of seeds removed by ants and damaged by slugs (total elaiosome removed) per transect at the
215 final observation time (24 hrs.). We included forest HLUC (remnant vs. secondary) and
216 ecoregion as fixed effects and site nested within ecoregion as a random effect to account for
217 spatial autocorrelation. We combined counts from two pitfalls per plot and performed a negative
218 binomial GLMM (to account for overdispersion) on *Aphaenogaster* sp. abundance, other ant
219 abundance, ant species richness. We estimated slug abundance as the combined number of slugs

220 (or slug evidence) on seed depots and slug abundance in pitfall traps. We performed a negative
221 binomial GLMM (to account for overdispersion) on slug abundance. Where ecoregion explained
222 variation for main response variables (seed removal, organism abundance), we performed a
223 Tukey's post hoc test to compare differences among regions. When there was HLUC*ecoregion
224 interactions, we compared remnant and secondary forests within ecoregions, performing a
225 Bonferroni correction for multiple comparisons (Appendix S1: Table S2).

226 We performed linear mixed effects models (LMM) on percent myrmecochore cover,
227 percent non-myrmecochore cover, shrub cover, tree basal area, and percent canopy openness at
228 the transect level. We log-transformed (myrmecochore cover, shrub cover, and total basal area)
229 to improve normality. We performed a negative binomial GLMM on species richness of
230 myrmecochores at the transect level. Finally, we performed principal component analyses (PCA)
231 on myrmecochore presence both at the site ($PCA_{S,myrmec}$) and transect level ($PCA_{T,myrmec}$) and
232 performed LMMs on $PC1_{T,myrmec}$ and $PC2_{T,myrmec}$. Where ecoregion explained variation for
233 myrmecochore richness and $PC1_{T,myrmec}$, we performed a Tukey's post hoc test to compare
234 differences among regions. When there was HLUC*ecoregion interactions, we compared
235 remnant and secondary forests within ecoregions, performing a Bonferroni correction for
236 multiple comparisons (Appendix S1: Table S2).

237 We also performed LMMs on soil pH, moisture, temperature and on average air
238 temperature at the transect level. We performed LMMs on rock surface area, leaf volume, and
239 log volume, performing log-transformations on log volume and rock area.

240 To determine how organisms interacting with seeds and habitat factors influence
241 observed variance in seed removal (See *Results*, Fig. 1), we performed path analyses. We
242 constructed an *a priori* model with hypothesized pathways determined by known ecological
243 interactions that included direct interactions between invertebrate organisms potentially
244 interacting with seeds (*Aphaenogaster* sp., other ants, and slugs) and seed removal (Appendix
245 S1: Fig. S5). We included direct interactions between "habitat" factors and the abundance of
246 organisms interacting with seeds, and an indirect interaction between habitat and seed removal.
247 We included direct interactions between other ants and *Aphaenogaster* sp. as other ants can
248 affect seed dispersal if they interact antagonistically with *Aphaenogaster* sp. (Ness 2004; Warren
249 et al. 2020). To maintain path analysis power, we created a composite habitat metric.

250 To create a composite habitat metric, we performed a correlation analysis among
251 standardized variables representing forest structure, abiotic factors, ant habitat factors, organisms
252 interacting with seeds (*Aphaenogaster* sp., other ant, and slug abundance), and seed dispersal
253 (Appendix S1: Fig. S8). We performed a PCA on habitat factors with significant correlations to
254 organisms and dispersal. $PC_{DM,hab}$ was the first component and explained 35.4 percent of
255 variation, and we used this component as the composite metric (Appendix S1: Fig. S10).
256 Similarly, we ran seed dispersal path analyses separately for remnant forests and secondary
257 forests and created composite habitat metrics for each ($PC_{DR,hab}$ and $PC_{DS,hab}$). We ran path
258 analyses on myrmecochore cover for the combined dataset and separately for remnant and
259 secondary forests (Appendix S1: Fig. S6). Myrmecochore cover was not significantly correlated
260 with seed removal (see details in Appendix S1).

261 We used R for all analyses, including the following packages: MASS, lme4, stats,
262 corrplot, lavaan, semplot, ggbiplot (Venables & Ripley 2002; Vu 2011; Rosseel 2012; Bates et
263 al. 2015; Epskamp 2019; R Core Team 2021; Wei & Simko 2021).

264
265

266 **Results**

267 Seed removal and invertebrate organisms interacting with seeds

268 The number of seeds removed from depots was significantly higher in remnant forests
269 compared to secondary forests ($P < 0.001$, Fig. 1A; see full statistical table, Appendix S1: Table
270 S2). During the seed removal trials, the majority ($> 90\%$) of ants removing seeds was
271 *Aphaenogaster* sp. We also observed 9 ant species interacting with (but not removing) seeds
272 (Appendix S1: Table S4). At 7 sites, we found *Nylanderia flavipes* (invasive ant) removing
273 pieces of elaiosomes from seeds. The other main organism on depots was *A. subfuscus*, that fully
274 scoops out elaiosomes, while not dispersing seeds (Meadley Dunphy et al. 2016).

275 In the pitfall traps, *Aphaenogaster* sp. abundance did not differ between forest HLUC
276 type ($P = 0.115$; Fig. 1C). The abundance of other ant species also did not differ, but there was a
277 trend for higher mean abundances in secondary forests ($P = 0.057$; Fig. 1D). We found 37 ant
278 species, with no difference in richness between remnant and secondary forests ($P = 0.783$;
279 Appendix S1: Table S2). Slug abundance was higher in secondary forests ($P = 0.0025$; Appendix
280 S1: Table S2). We also found the number of seeds remaining with their elaiosome fully removed
281 (due to slug damage) was higher in secondary forests ($P = 0.0176$; Fig. 1B). We observed
282 variation in seed removal, ant richness, and ant abundance among regions but no variation in
283 slug abundance (Appendix S1: Table S2).

284

285 Myrmecochore diversity and vegetation structure

286 We found 28 myrmecochore species (Appendix S1: Table S3), with total myrmecochore
287 cover ($P = 0.0316$; Fig. 3A) and myrmecochore species richness being lower in secondary forests
288 ($P = 0.0041$; Fig. 3B). In the site level myrmecochore PCA, we found $PC1_{S.myrmec}$, accounted for
289 25.3% of variance with more forest indicator species at remnant sites, including *Dicentra* sp.
290 (including *D. cucullaria*), *Tiarella cordifolia*, *Trillium* sp. (including *T. erectum*) along with
291 nonsignificant indicator species *Anemone acutiloba* and *Claytonia caroliniana* (Appendix S1:
292 Table S3). $PC2_{S.myrmec}$ accounted for 16.1% of variance and was influenced by indicator species
293 *T. grandiflorum*, *T. undulatum*, and *Uvularia sessilifolia* (Griffiths & McGee 2018) (Fig. 2). We
294 found a difference in $PC1_{T.myrmec}$ by forest HLUC ($P = 0.0328$; Appendix S1: Table S2), but
295 $PC2_{T.myrmec}$ did not differ between forest HLUC (Appendix S1: Fig. S3). Canopy openness was
296 higher in secondary forests ($P = 0.0084$; Fig. 3C), but there were no differences in shrub cover,
297 non-myrmecochore herbaceous understory cover, or tree basal area between forest types
298 (Appendix S1: Table S2). There were ecoregion effects for myrmecochores, with myrmecochore
299 richness being higher in E3 than in E1 and E2 (Fig 3B; Appendix S1: Table S2).

300

301 Abiotic characteristics and potential ant habitat

302 We did not find differences in average soil temperature, soil pH, soil moisture, or air
303 temperature between HLUC (Appendix S1: Table S2). We also found no differences in leaf litter
304 volume, log volume, or rock surface area between HLUC (Fig. 3D). Across both types of HLUC,
305 we found a slight difference in habitat types occupied by ant colonies, preferring log over leaf
306 litter and rock (Appendix S1: Fig. S4).

307

308 Path analyses and correlation analyses

309 In the combined seed dispersal path model, we found significant and positive interactions
310 between *Aphaenogaster* sp. abundance and other ant abundance (0.72 ± 0.16 ; $P < 0.001$; Fig.
311 4A). *Aphaenogaster* sp. abundance had a significant positive effect on seed removal ($0.47 \pm$

312 0.16; $P < 0.01$) and slug abundance had a significant negative effect (-0.36 ± 0.11 ; $P < 0.01$). The
313 abundance of other ants had no effect on seed removal (Fig. 4A). $PC_{DM.hab}$ had a significant
314 negative effect on the abundance of other ants (0.38 ± 0.12 ; $P < 0.01$; Fig. 4A). Given the factors
315 that contributed to $PC_{DM.hab}$, ant abundance is higher at sites with relatively lower soil moisture,
316 leaf litter, and air temperature, and higher soil pH, soil temperature, and herbaceous cover (Fig.
317 4A; Appendix S1: Fig S8). The final model was supported by the data ($\chi^2 = 1.40$; d.f. = 2; $P =$
318 0.49). A P value > 0.05 suggests the data are consistent with the hypothesized model.

319 The remnant forest path model also revealed that *Aphaenogaster* sp. abundance and other
320 ant abundance had a significant, positive interaction (0.80 ± 0.23 ; $P < 0.01$; Fig. 4B). However,
321 in this case, the abundance of organisms had no significant direct effect on seed removal. In the
322 remnant path, $PC_{DR.hab}$ had significant effects on *Aphaenogaster* sp. and other ants (0.46 ± 0.16 ,
323 $P < 0.01$; 0.59 ± 0.15 , $P < 0.001$; Fig. 4B), again with soil conditions (lower moisture and higher
324 temperature) influencing ant abundance (Appendix S1: Fig. S8). The secondary forest path
325 model revealed that *Aphaenogaster* sp. abundance had a direct positive effect on seed removal
326 (0.39 ± 0.15 ; $P < 0.05$; Fig. 4C), but no other interactions were significant. Secondary $PC_{DS.hab}$
327 had no effect on organism abundance (Fig. 4C; Appendix S1: Fig. S8). The final models were
328 both supported by the data ($\chi^2 = 1.01$, d.f. = 2, $P = 0.60$; $\chi^2 = 1.98$, d.f. = 2, $P = 0.37$). The
329 combined and separate path analyses showed weak effects of organisms' abundance and habitat
330 factor impacts directly on myrmecochore cover (Appendix S1: Fig S6). However, our correlation
331 analysis shows significant positive effect of soil pH and other herbaceous (non-myrmecochore)
332 cover on myrmecochore cover in the combined and remnant forests, but not the secondary
333 forests (Appendix S1: Fig. S9).

334

335 Discussion

336 Our work shows that seed dispersal by ants, a vital ecosystem function in NAEDF
337 ecosystems, is partially resilient to disturbances from HLUC with variation in recovery
338 trajectories. Secondary forests had lower and more varied rates of seed dispersal than remnant
339 forests, resulting from altered interactions with mutualists and antagonists. High-quality
340 mutualists, *Aphaenogaster* sp., were the primary dispersers of seeds and variation in their
341 abundance contributed to variation in seed dispersal. Other ant species did not affect seed
342 dispersal, but invasive slugs were more abundant and slug-induced seed damage more prevalent
343 in secondary forests, with a negative relationship between slug abundance and seed dispersal.
344 Interestingly, variation in *Aphaenogaster* sp. abundance did not influence seed dispersal in
345 remnant forests (only in secondary forests), suggesting that seed dispersal function is stable in
346 remnant forests, but variable in secondary forests, where dispersal quality is dependent on
347 *Aphaenogaster* sp. abundance. Myrmecochore cover and richness was lower in secondary
348 forests, but not influenced by *Aphaenogaster* sp. abundance. Taken together, secondary forests
349 vary in functional resilience, where dispersal is reduced in some forests with low mutualist
350 abundance, but intact where mutualists are abundant. While variation in functional resilience
351 may not be a direct mechanism of low recovery of myrmecochory diversity in secondary forests,
352 animal-mediated seed dispersal, including myrmecochory, is known to be important for plant
353 fitness, distribution, and community structure (Kalisz et al. 1999; Canner et al. 2012; Prior et al.
354 2015). As result, functional resilience of seed dispersal interactions should be considered when
355 restoring myrmecochore communities.

356 We predicted that seed dispersal would be lower in secondary forests due to lower
357 abundances of mutualistic partners, *Aphaenogaster*. While we found lower rates of seed dispersal

358 in secondary forests, *Aphaenogaster* sp. was present in all forests, with no difference in
359 abundance between HLUC. This suggests that *Aphaenogaster* are somewhat resilient to
360 widespread forest clearing. Similarly, previous work found no difference in abundances of
361 *Aphaenogaster* between forests of differing HLUC (Mitchell et al. 2002; Kiel et al. 2020).
362 Despite finding no difference, variation in *Aphaenogaster* sp. abundance was the primary
363 determinant of seed dispersal variation in all forests. Interestingly, the sensitivity of seed
364 dispersal to mutualist abundance differed between remnant and secondary forests. In remnant
365 forests, seed dispersal variation was not influenced by *Aphaenogaster* sp. abundance, but
366 dispersal in secondary forests was sensitive to changes in *Aphaenogaster* sp. abundance. These
367 different relationships suggest that seed dispersal function is stable and intact in remnant forests,
368 but variable in secondary forests, with high dispersal occurring in secondary forest patches with
369 high *Aphaenogaster* sp. abundance.

370 In our combined and remnant seed dispersal path analysis, we found an effect of habitat
371 conditions on other ant and *Aphaenogaster* sp. abundance. Our findings support previous studies
372 that show that *Aphaenogaster* distribution is determined by microhabitat conditions, specifically
373 that they do not occupy soil with high moisture (Warren et al. 2010; 2011; 2012). In secondary
374 forests, *Aphaenogaster* sp. abundance was not influenced by habitat variation. This could be due
375 to greater microhabitat homogenization, a legacy of widespread forest clearing (Flinn & Marks
376 2007). Additionally, variation in nest disturbance or recolonization ability from local source
377 populations may be more important in influencing abundance than microhabitat conditions.

378 We also predicted variation in antagonists would influence seed dispersal and found
379 higher abundances of *A. subfuscus*, and higher rates of slug-caused seed damage in secondary
380 forests. In the combined path analysis, we found a negative relationship between slugs and seed
381 dispersal. Elaiosome removal by slugs decreases dispersal by ants by removing the attractive
382 food reward and disrupting the interaction (Meadley Dunphy et al. 2016). Slugs might be more
383 abundant in secondary forests if they have increased access through forest fragmentation and
384 proximity to other habitats, like old fields (Beyer & Saari 1978; Kozłowski 2009). In a previous
385 study, we found higher slug abundances and elaiosome damage at forest edges than in forest
386 interiors (Parker et al. 2021). Slug abundance might also be higher in secondary forests due to
387 changes in environmental conditions, but we found few consistent differences in microhabitat
388 conditions between HULC. We found no effect of habitat factors on slug abundances in our path
389 analyses, but soil temperature had a positive correlation with slug abundance, and there was a
390 negative relationship with soil moisture (Appendix S1: Fig. S8). Future studies investigating the
391 mechanisms leading to increased slug presence in secondary forest would be useful, especially
392 when considering restoring understory plants reliant on seed dispersal mutualisms.

393 In all of our path analyses, other ant species (species other than *Aphaenogaster* sp.)
394 abundance did not have a direct effect on seed dispersal but had a strong positive interaction with
395 *Aphaenogaster* sp. abundance. This suggests that overall, other ant species do not have a direct
396 antagonistic interaction with seeds or mutualist partners. This is an interesting finding, as it is
397 generally predicted that other ants negatively affect dispersal directly (by being antagonistic or
398 low quality partners) or indirectly by outcompeting the good disperser (Ness 2004; Giladi 2006;
399 Ness et al. 2009; Prior et al. 2020; Parker et al. 2021). Habitat factors in both the combined and
400 remnant path analysis influenced other ant species abundance, which suggests that the other ant
401 species were more abundant in microhabitats that also favored *Aphaenogaster* sp.. During the
402 seed removal trials, we only observed two other ant species interacting with, but not removing,
403 seeds: the native species *Lasius americanus* and the invasive species *Nylanderia flavipes*, with

404 the latter occurring at high abundances and removing parts of elaiosomes. Taken together, other
405 ants do not seem to be largely antagonistic, but also do not always contribute to seed dispersal
406 function.

407 Previous work examining HLUC on myrmecochory in NAEDF found that patch size and
408 historical land use intensity influences ant community abundance and composition (Mitchell et
409 al. 2002). While this previous study found little effect of HLUC on *Aphaenogaster* abundance,
410 they did not quantify how the abundance or presence of ants directly affected seed dispersal
411 function. More recent work directly assessing seed removal by ants in forests that differed in
412 HLUC demonstrated that rates of removal did not differ (Kiel et al. 2020). While this work
413 contributes to our understanding of HLUC effects on ant-mediated seed dispersal, it was limited
414 in spatial scope, not accounting for variation at the landscape level, with only three forests in a
415 narrow portion of the range of this mutualism. Here, we covered a larger portion of this
416 mutualism's range and in doing so demonstrated variation in functional resilience at the
417 landscape level. We also found ecoregion effects on ant abundances, forest structure, and abiotic
418 factors which could contribute to variation in functional resilience across space. To this end our
419 study does not contradict either previous study, rather our increased scale and direct
420 measurement of seed dispersal function expands the scope, revealing variation in functional
421 resilience despite *Aphaenogaster* being present in secondary forests.

422 We found that total myrmecochore cover and richness were lower in secondary sites.
423 This finding is similar to previous work that finds lower abundances of myrmecochore species in
424 forests that have been previously cleared (Bellemare et al. 2002; Mitchell et al. 2002; Vellend
425 2005; Griffiths & McGee 2018). In our myrmecochore PCA, myrmecochore species composition
426 differed between secondary and remnant forests. Remnant forests had more cover and species
427 richness, and particularly higher presence and abundance of specific remnant forest indicator
428 species (Griffiths & McGee 2018). We found that myrmecochore cover was not influenced by
429 *Aphaenogaster* sp. abundance in our combined path analysis and that myrmecochore cover and
430 seed dispersal is not significantly correlated. This suggests that factors other than ant mutualists
431 and seed dispersal are contributing to variation in myrmecochore cover. Habitat factors such as
432 soil pH, nutrient availability, and organic matter content are all known to be altered by forest
433 clearing and agricultural disturbances and could be contributing to lower myrmecochore cover in
434 secondary forests (Koerner et al. 1997; Dyer 2010). However, we found no difference in soil
435 characteristics between forests with different HLUC which suggests soil resilience in some
436 disturbed forests. We found secondary forests had higher canopy openness, which could impact
437 other abiotic factors that contribute to myrmecochore cover such as light availability. Other
438 studies suggest that low myrmecochore richness and cover may also be affected by recruitment
439 limitation from source populations (Bellemare et al. 2002; Flinn & Vellend 2005).

440 One consideration in our study is that forests varied in myrmecochore cover, which could
441 affect seed dispersal. For example, *Aphaenogaster* colonies are known to become satiated with
442 myrmecochorous seeds (Heithaus et al. 2005), and forests with more myrmecochores could mean
443 that ants forage less for seeds. However, we found no relationship between myrmecochore cover
444 and seed dispersal. Another source of variation we did not account for, is that we pooled
445 *Aphaenogaster* species given that they are challenging to tell apart in the field. Emerging work,
446 including our own, suggests that mutualistic partner identity (among *Aphaenogaster* putative
447 species and populations) can affect seed dispersal function (Warren & Bradford 2014; Prior et al.
448 2015; Meadley Dunphy et al. 2020; Prior et al. 2020; Buono et al. 2022). Also, we controlled for

449 rodent impacts on seed dispersal, but variation in this antagonistic interaction could also be
450 contributing to how HLUC affects this mutualism (Ness & Morin 2008).

451 Variation in legacy effects and resilience can cause variable recovery trajectories, making
452 predicting resiliency or trying to reverse impacts of disturbances difficult and complex (Suding
453 et al. 2004). Specifically for ant-mediated seed dispersal in NAEDF, while mutualist ant partners
454 are present in secondary forests, suggesting some level of resilience, there is variation in their
455 abundance and function. Other studies show variation in the resiliency of diversity post
456 disturbance (Steadman 1997; Elmquist et al. 2002; Sabatini et al. 2014), but less is known about
457 the resiliency of functionally important interactions that diversity relies on (Oliver et al. 2015,
458 (Mitchell et al. 2002; Kaiser-Bunbury et al. 2017; García et al. 2018). Uncovering how
459 interactions that species rely on are resilient to disturbance is critical to understand mechanisms
460 of slow recovery or predict if functions will be intact for proposed active restoration.

461 Our research provides implications for restoration efforts. First, we emphasize the
462 importance of preserving remaining remnant forest ecosystems to provide critical source
463 populations for recovery. Second, given that not all secondary forests are resilient to historical
464 forest clearing suggests that forest patches with intact seed dispersal interactions might be
465 prioritized for active restoration of understory plants, or there may need to be efforts to augment
466 or enhance this interaction in some forests. While the presence of seed dispersal function and
467 mutualistic ants do not directly determine plant community resilience, their documented
468 importance on understory plant populations and communities means that maintenance of this
469 function will be essential to conserving understory plant communities.

470

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485 these regions, and we hope to advocate for increased Indigenous recognition within the scientific
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487

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489 KMP, CG, and JS conducted the fieldwork; CMB, JF, WS, CG, and JS performed lab-work;
490 CMB analyzed the data with input from KMP; CMB wrote the manuscript with input from KMP.

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680
681

682 **Figure captions**

683

684 **Figure 1.** A) Seeds removed from depots, B) seeds with slug damage, C) *Aphaenogaster* sp.
685 abundance, and D) other ant species abundance between remnant and secondary forests at the
686 transect level (n = 60). Thick lines in box plots represent medians, boxes represent 1st and 3rd
687 quartiles, whiskers represent minimums and maximums, and points represent outliers.

688

689 **Figure 2.** Myrmecochore species site level biplot of principal component analysis (PCA).
690 Remnant transects (r) represented by green symbols and secondary (s) are tan symbols. Remnant
691 sites are more diverse (spread out) compared to the majority of secondary sites clustered on the
692 right side of PC1_{S,myrmec}. PC1_{S,myrmec} explains 25.3% of variance and PC2_{S,myrmec} 16.1%. Black
693 arrows represent indicator species and gray arrows nonsignificant indicator species (Griffiths &
694 McGee 2018). Species acronyms found in Appendix S1: Table S3.

695

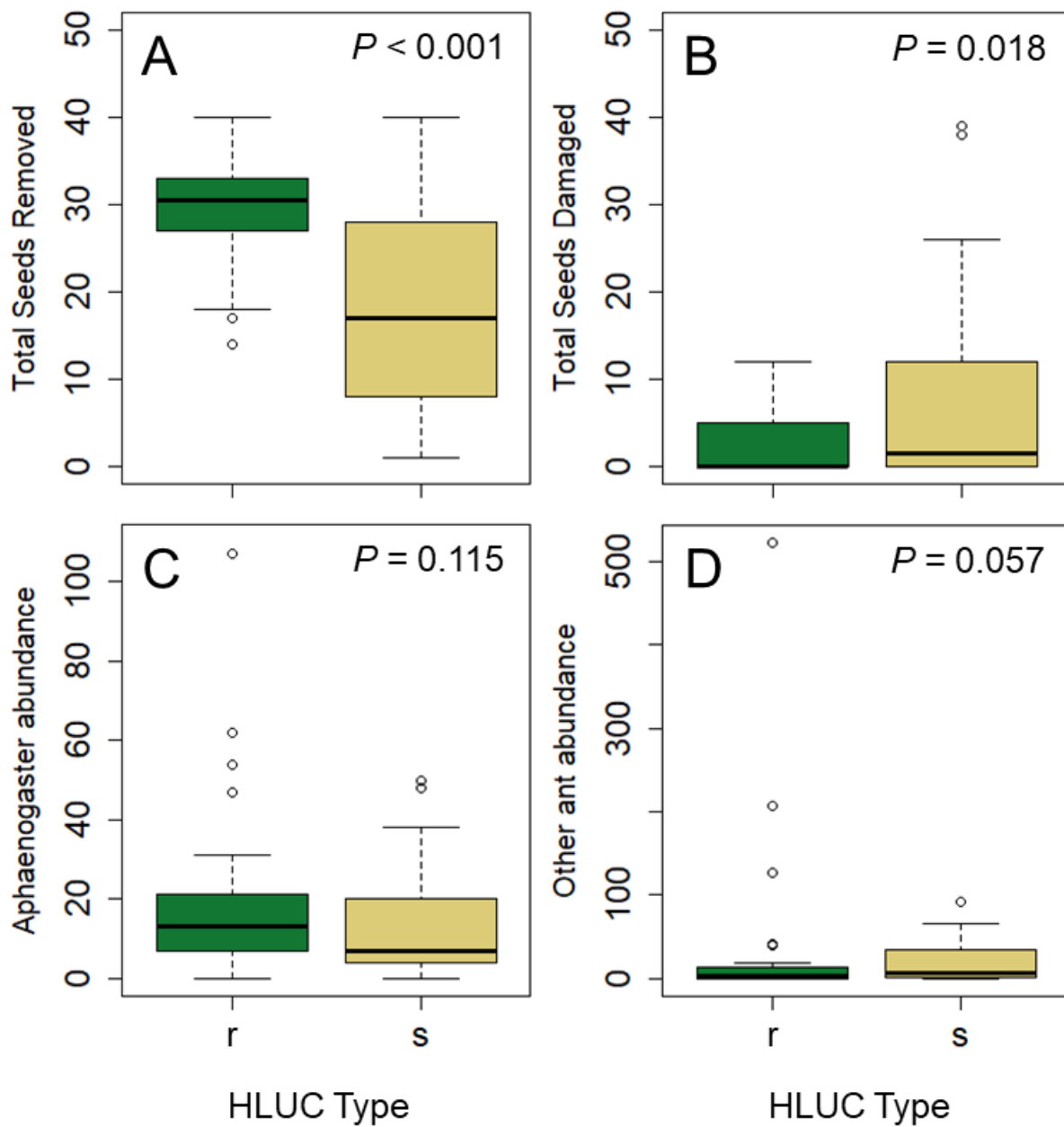
696 **Figure 3.** A) Myrmecochore species cover, B) myrmecochore species richness, C) canopy
697 openness, D) log habitat volume at the transect level (n = 60) in remnant (r) and secondary (s)
698 forests. Thick lines in box plots represent medians, boxes represent 1st and 3rd quartiles,
699 whiskers represent minimums and maximums, and points represent outliers.

700

701 **Figure 4.** Seed dispersal path diagram with standardized path coefficients reported next to the
702 arrows for A) all forests (combined) B) remnant forests and C) secondary forests. Green and tan
703 solid arrows indicate significant positive and negative pathways respectively. Thickness of
704 arrows are proportional to the standardized path coefficient's strength. Non-significant pathways
705 with path coefficients less than 0.1 are given in dashed gray lines. Significant differences
706 represented by symbols (* > 0.05, ** > 0.01, *** > 0.001).

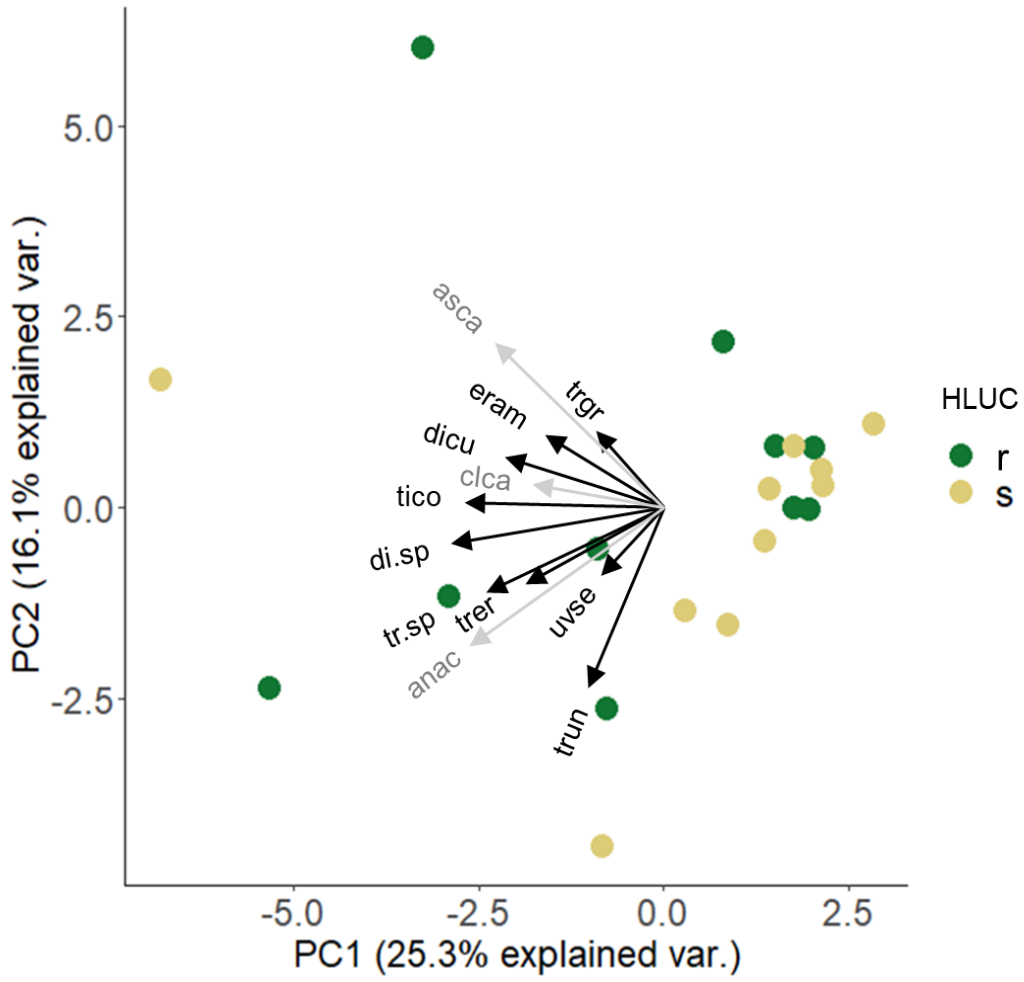
707

708 **Figure 1**



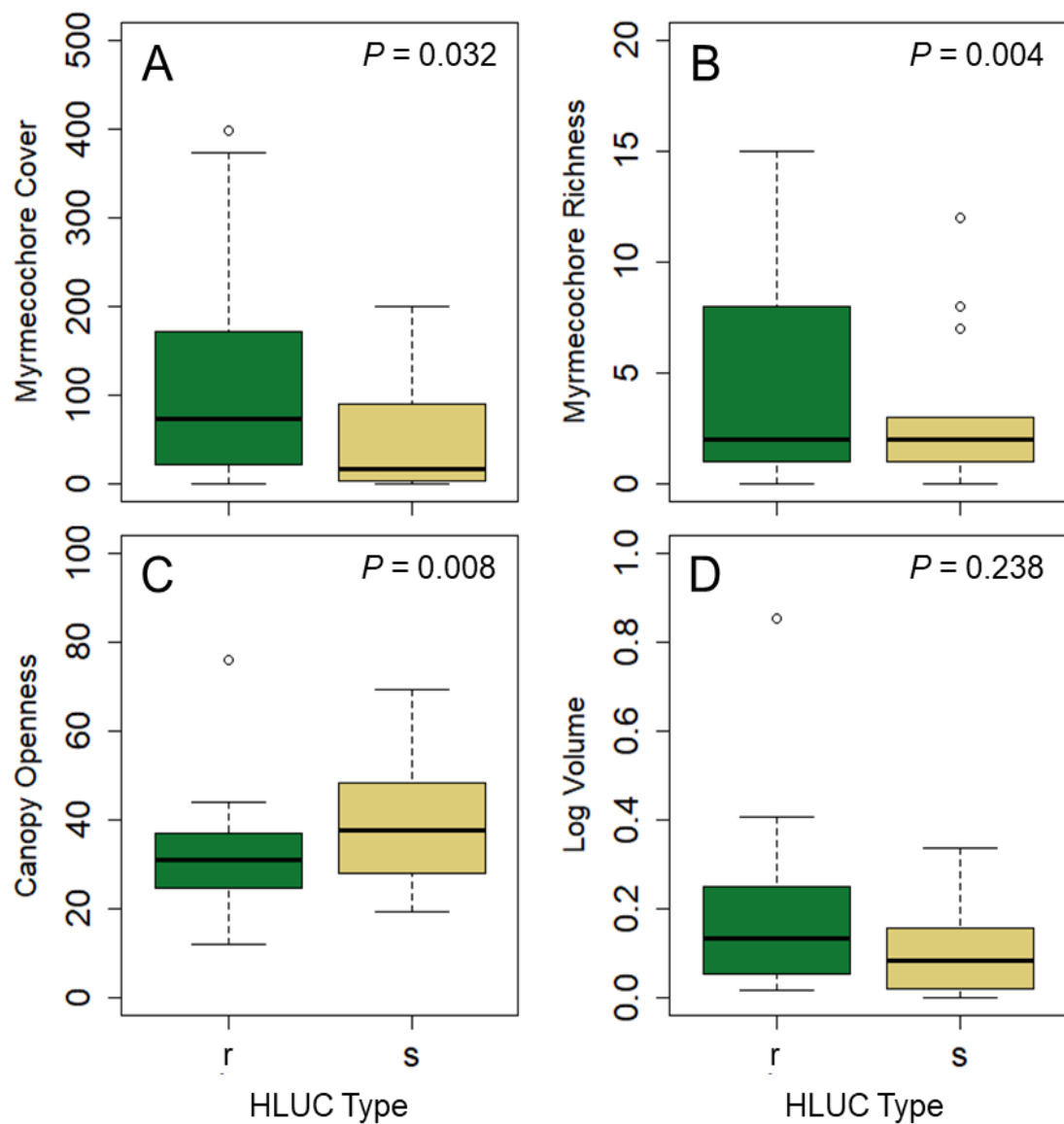
709
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711 **Figure 2**



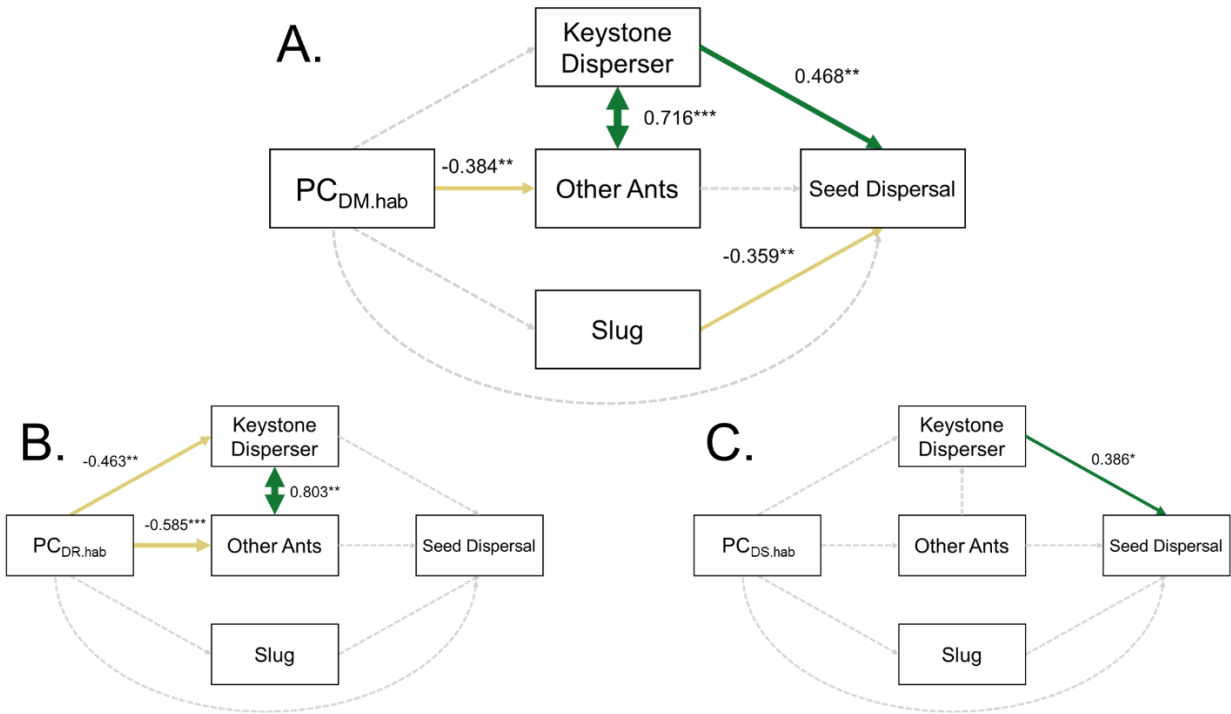
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714 **Figure 3**



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716 **Figure 4**



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