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1 **Running title:** Disturbance reshapes diaspore interactions

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4 **Topsoil disturbance reshapes diaspore interactions with**
5 **ground-foraging animals in a megadiverse grassland**

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7

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20

21 **Abstract**

22 *Questions*

23 Anthropogenic disturbances are known to be followed by extremely poor recovery in edaphic
24 grasslands. However, the role of interactions with diaspore predators and secondary dispersers,
25 which compose the dispersal filter and modulate plant community recovery, remains
26 overlooked. We performed field experiments to investigate how soil disturbances affect
27 diaspore interactions with the ground-foraging fauna to better understand how disturbance
28 influences regeneration potential.

29 *Location*

30 *Campo rupestre* vegetation, megadiverse edaphic grasslands, southeastern Brazil.

31 *Methods*

32 We used diaspores from five native species to compare removal rates between disturbed (topsoil
33 removal) and preserved sites. We controlled invertebrate and vertebrate access to determine
34 their role. Additionally, we assessed differences in the diaspore removal effectiveness (DRE)
35 and the structure of animal-diaspore interactions through network based-approach.

36 *Results*

37 For three species, diaspore removal was relatively high (between 50 and 100%). Invertebrates
38 were the most common removal agents in both disturbed and preserved sites. Interactions with
39 foraging fauna and removal rates were 20% smaller in disturbed sites. Considering all diaspores
40 removal events in disturbed sites, 24% resulted in the displacement from disturbed to preserved
41 sites, but no diaspore was transported from preserved to disturbed ones. The animal-diaspore
42 network was exclusively composed by ant-diaspore interactions and were more diverse and
43 robust in preserved sites compared to disturbed ones. Seed predator ants (*Pheidole* and
44 *Dorymyrmex*) were more common in disturbed sites. Furthermore, we found significant
45 differences in the DRE between ant species and site types, suggesting specificity in the
46 provision of dispersal services.

47 *Conclusions*

48 Topsoil removal affected removal proportions, DRE and ant-diaspore interaction network
49 structure. The lack of diaspore dispersal towards disturbed sites indicates that soil removal
50 affects secondary seed removal dynamics, precluding recovery potential. Disturbance
51 negatively affected diaspore fate by reshaping interactions with ground-foraging secondary
52 seed dispersers and predators, constraining the development of seed bank and thus vegetation
53 dynamics and resilience.

54

55 Keywords: ants, *campo rupestre*, degradation, diaspore removal, myrmecochory, natural
56 recovery, seed limitation, seed predation

57

58 **Introduction**

59 Ground-foraging animals may affect seed fate by interacting with diaspores handled or
60 dispersed by primary dispersers (Roberts & Heithaus 1986; Chambers & MacMahon 1994;
61 Vander Wall et al. 2005). Empirical studies examining secondary diaspore-animal interactions
62 have shown a strong variability among plant species and habitat types (Schupp & Fuentes 1995;
63 Chapman & Chapman 1996, Lambert 2002; Roselli 2014), and have been crucial to the
64 development of theoretical models regarding seed fate (Janzen 1970, Hammond & Brown 1998;
65 Martinson & Fagan 2014; Török et al. 2018). By moving seeds, ground-foraging animals may
66 expand seed shadows generated by primary dispersers (Christianini & Oliveira 2009), provide
67 additional opportunity for seeds to escape predation or fire (Giladi 2006; Rico-Gray & Oliveira
68 2007; Rico-Gray & Oliveira 2007), and in some cases lead to direct seed deposition into
69 nutrient-rich soils (Sternberg et al. 2007, Arnan et al. 2012). On the other hand, seed removal
70 can result in seed loss by predation or deposition in unfavorable sites, affecting plant
71 community assembly and recovery in disturbed areas (Calviño-Cancela 2007; Denham 2008).

72 Diaspore-animal interactions in tropical ecosystems often involve different agents of
73 dispersal in subsequent steps (Vander Wall & Longland, 2004; Camargo et al. 2019). Secondary
74 seed dispersal has been commonly attributed to vertebrates, such as rodents (Feer & Forget
75 2002; Lessa et al. 2013; Genrich et al. 2017). However, the contribution of invertebrates as
76 secondary diaspore dispersers still remains poorly understood (Magalhães et al. 2018; Camargo
77 et al. 2019). Ants are well-known as seed predators (Retana et al. 2004; Costa et al. 2017), but
78 they can also play an important role as seed dispersers in Neotropical savannas (Christianini &
79 Oliveira 2009, 2010), often improving seed survival and germination by feeding upon fruit pulp

80 and cleaning seeds, which become less vulnerable to pathogen attack (Passos & Oliveira 2003;
81 Christianini et al. 2007). Even though most diaspores in Neotropical savannas do not present
82 any apparent characteristics that promote ant dispersal (Christianini & Oliveira 2010),
83 evidences suggest ants as important seed dispersers of non-myrmecochorous plant species (e.g.,
84 Leal & Oliveira 1998; Christianini et al. 2007; Christianini & Oliveira 2009, 2010; Lima et al.
85 2013; Guerra et al. 2018).

86 The Brazilian *campo rupestre*, an Old Climatically-Buffered Infertile Landscape
87 (OCBIL *sensu* Hopper et al. 2016), encompasses old-growth fire-prone tropical grasslands,
88 associated to extremely poor soils on ancient mountaintops that harbors a highly diversified
89 flora with remarkable levels of plant endemism (Echternacht et. al. 2011; Silveira et al. 2016;
90 Colli-Silva et al. 2019). Nevertheless, along the past decades, the vegetation dynamics in these
91 unique landscapes has been hampered by anthropogenic disturbances, especially topsoil
92 removal associated with quarrying and mining activities (Silveira et al. 2016; Fernandes et al.
93 2018). Plant and ant communities from *campo rupestre*, although highly resilient to endogenous
94 disturbances, such as fires (Fagundes et al. 2015; Neves et al. 2016; Le Stradic et al. 2018a),
95 are extremely vulnerable to human-induced exogenous soil disturbances (Le Stradic et al.
96 2018b; Buisson et al. 2019), which can be partially explained by dispersal limitation (Morellato
97 & Silveira 2018).

98 In old-growth grasslands, plant species disperse poorly and at low rates, making
99 community re-assembly a lengthy process (Bond & Parr 2010; Parr et al. 2014; Veldman et al.
100 2015; Buisson et al. 2019). In seed-limited ecosystems, such as *campo rupestre*, reductions in
101 seed quantity (e.g., seed predation) may compromise recruitment, while processes that increase
102 seed dispersal success may prompt it (Calviño-Cancela 2007). Nevertheless, only a handful of
103 studies have addressed the influence of diaspore-animal interactions on vegetation dynamics in

104 *campo rupestre* (Lima et al. 2013; Guerra & Pizo 2014, Guerra et al. 2016, 2017, 2018), and
105 none have addressed this topic within a disturbance context.

106 Here, we aimed to ascertain how topsoil disturbance affects diaspore interaction with
107 ground-foraging fauna in *campo rupestre*. First, we compared the proportion of diaspores
108 removed for five plant species between disturbed and preserved sites using control and
109 vertebrate-exclosure treatments (to differentiate the role of vertebrates and invertebrates in
110 diaspore removal). Then, we qualitatively compared diurnal animal-diaspore interactions
111 between disturbed and preserved sites for diaspores of three plant species that had significant
112 removal rates in the first experiment. As ants were the only group observed in this second
113 experiment, we recorded ant-diaspore interactions and explored their possible outcomes
114 through multilayer networks. Finally, we explored the effects of topsoil disturbances on
115 interaction structure and seed dispersal effectiveness among ant species in both disturbed and
116 preserved sites.

117 **Material and methods**

118

119 *Study area*

120 We conducted this study at the vicinity of the Serra do Cipó National Park, in the
121 southern portion of the Espinhaço Range, southeastern Brazil (43° 35'W, 19° 17'S). The annual
122 precipitation at the study site averages around 1,400 mm and the climate is markedly seasonal
123 with most rainfall concentrated between October and March (Brito et al. 2017). Altitude at the
124 study site ranges between 1,150 and 1,300 m a.s.l. The main vegetation at the study site is
125 *campo rupestre*, an old-growth, fire-prone grassland established on quartzite-derived rocks,
126 with shallow and severely nutrient-impoverished sandy soils in mountaintops (Veldman et al.
127 2015; Silveira et al. 2016, Mucina 2018). The landscape encompasses a mosaic formed by

128 patches of rocky outcrops and boulders where sclerophyllous treelets and shrubs grow and
129 patches of an herbaceous stratum, dominated by monocots and sparsely distributed shrubs (Le
130 Stradic et al. 2015; Guerra et al. 2017).

131 During the paving of the MG-010 highway in 2002, small quarries were exploited for
132 soil extraction, destroying vegetation and virtually removing all topsoil horizons. Owing to
133 disturbance, the community composition between preserved and disturbed sites remains
134 drastically different even 15 years after the disturbance (Le Stradic et al. 2018b). For the present
135 study, we selected four disturbed sites of at least 100 m² and a preserved (control) site nearby
136 each disturbed one. In all sites, the paired disturbed and preserved sites were adjacent at
137 approximately 30 meters away from each other and each pair distant at least 2 km from another.
138 The preserved sites are grasslands used as the reference ecosystem of the disturbed sites. The
139 disturbed sites have extensive areas of bare ground and a few grassland species. All permits to
140 visit and collect biological data were authorized by the Instituto Chico Mendes de Conservação
141 da Biodiversidade (ICMBio) of the Brazilian Ministry of Environment. Data collection in sites
142 located on private lands was authorized by the owners and ICMBio.

143 *Diaspores from native species*

144 We used diaspores from five native plant species commonly found in the study area,
145 which have similar likelihood of being dispersed to disturbed and adjacent preserved sites, to
146 compare diaspore removal and interactions with foraging animals between diaspore types and
147 site types (Appendix S1). We selected the species based on four criteria: (1) diaspore size; not
148 too small to prevent the diaspores from being blown away, (2) presence of fleshy parts or rich
149 endosperm to potentially attract both vertebrates and invertebrates, (3) diaspore availability; to
150 allow setting replicates for the removal experiments (between 600 to 1000 diaspores per
151 species), and (4) phylogenetic and morphological diversity. In our experiment, we used seeds

152 of *Stryphnodendron gracile* (Fabaceae) and *Davilla elliptica* (Dilleniaceae). Additionally, we
153 used berries of three species: *Miconia irwinii* (Melastomataceae), *Byrsonima vacciniifolia*
154 (Malpighiaceae) and *Coccoloba cereifera* (Polygonaceae), all primarily dispersed by birds (see
155 Guerra et al. 2016).

156 *Stryphnodendron gracile* (Fabaceae) is a Brazilian endemic small shrub, found
157 exclusively in the cerrado biome (Occhioni 1990). It typically produces seeds characterized by
158 a very rigid seed coat (De Lima 1985). *Davilla elliptica* (Dilleniaceae) is a widespread species
159 in the cerrado biome (Fraga 2012) and produces fruits containing up to two seeds surrounded
160 by an aril (Pott & Pott 1994). *Miconia irwinii* (Melastomataceae) is a Brazilian endemic treelet
161 found exclusively in rocky outcrops at *campo rupestre*, with single plants producing up to 4,000
162 water- and sugar-rich purplish-black berries that are primarily dispersed by birds (Guerra et al.
163 2017), and secondarily dispersed by ants and lizards, but being also predated by ground
164 dwelling ants and rodents (Guerra et al. 2018). *Byrsonima vacciniifolia* (Malpighiaceae) is a
165 Brazilian endemic treelet found exclusively in the cerrado and caatinga biomes (Mamede &
166 Francener 2015), which typically produces water- and sugar-rich yellow fruits with a striking
167 aroma (Leal et al. 2007). *Coccoloba cereifera* (Polygonaceae) is a narrowly distributed endemic
168 species from *campo rupestre* sandy grasslands in southeastern Brazil (Ribeiro & Fernandes
169 1999), which typically produces fleshy violet fruits. We collected all native diaspores during
170 the fruiting period of each species and set them in the refrigerator (4°C) until the
171 commencement of each experiment.

172 *Removal experiment design*

173 Experiment A. We performed randomized block factorial experiments to compare
174 diaspore removal rates between disturbed and preserved sites, and between vertebrate-exclosure
175 and control treatments. In each sampling station, we used either wire frames to create a

176 treatment accessible to invertebrates and vertebrates (control treatment) or wired cages to create
177 treatments to exclude vertebrates, but accessible to invertebrates (vertebrate-exclosure
178 treatment; Appendix S2). We constructed the wire cages ($17 \times 17 \times 8$ cm) fenced with wire
179 mesh (1.2 cm). The wire frames were constructed without mesh (i.e., access to both vertebrates
180 and invertebrates) and were used to control possible effects of wire presence on diaspore
181 removal (Guerra et al. 2018). We performed five experiments separately, one for each species,
182 simultaneously in all sites for each species. We paired our removal stations in six blocks
183 distributed along four disturbed and four preserved adjacent sites. In each block, we thus set
184 paired control (wire frame) and vertebrate-exclosure (wire cage) treatments, totaling 96 samples
185 per plant species (experimental design shown in Appendix S2). Blocks were distant nearly 1 m
186 from each other.

187 We conducted the experiment in the summer from December 2016 to March 2017, the
188 period of higher activity by ground-foraging animals (Costa et al. 2018; Pol et al. 2011). The
189 sampling effort for each species occurred during the same week within this 3-month interval.
190 Each sampling station consisted of diaspore piles placed in the ground over a filter paper,
191 always controlling for the number of diaspores for each species. No rain or strong winds
192 occurred during the observation periods, reducing the possibility that seeds were lost due to
193 abiotic factors. Using the filter paper was important to gather and count diaspore during
194 experiments. We used white filter papers considering this the less contrasting color with the
195 white sandy soil in the study sites. Any possible contrast effects by the filter paper leading to
196 attraction or deterrence of animals is present in all treatments.

197 We placed 10 diaspores per sampling station totaling 960 diaspores per species, with
198 the exception of *Byrsonima* and *Davilla*, in which we placed eight and six diaspores per
199 sampling station, totaling 768 and 576 diaspores, respectively. We evaluated the proportion of
200 diaspores removed in all treatments by exhaustively searching for diaspores in stations after

201 48h of exposure in the field (Guerra et al. 2018). The proportion of diaspores removed (PDR)
202 from each sampling station was calculated as:

$$203 \quad PDR = (Nrc \text{ or } Nre)/No$$

204 where *No* is the number of offered diaspores, and *Nrc* and *Nre* are the number of
205 recovered diaspores in the control treatment and in the vertebrate-exclosure treatment,
206 respectively.

207 Experiment B. To determine the identity of animal species interacting with diaspores,
208 the frequency of interactions, and their behavior towards the diaspores, we performed direct
209 diurnal observations on diaspore piles in disturbed and preserved sites. To do that, we used
210 diaspore of the three species that presented the most significant removal rates in the first
211 experiment: *Byrsonima vacciniifolia*, *Davilla elliptica* and *Miconia irwinii*.

212 For these species, we performed direct observations bouts totaling 20 hours for each
213 species, equally distributed between the four disturbed and preserved sites. We obtained and
214 handled the diaspores as described above, placing five diaspores of each species directly on the
215 ground over a filter paper, without any structure around. We performed the observation bouts
216 during the daytime, always between 10:00 AM and 05:00 PM. Each bout comprised 50 minutes
217 of continuous observation performed by a single observer. We followed the animals that
218 effectively removed diaspores from piles, recorded dispersal distances, the final destination
219 (e.g., ant nest and preserved or degraded site), and animal behavior towards the diaspore
220 whenever possible. Ants were the only group of animals observed interacting with diaspores.
221 Interacting ant specimens were collected, preserved in 70% alcohol, and identified to the lowest
222 possible taxonomic level using the taxonomic key provided by Baccaro et al. (2015). Collected
223 specimens were also compared to a reference collection from the Insect Ecology Lab at UFMG
224 (Costa et al. 2016), in order to ensure species identification. Ant behavior towards diaspores
225 was classified as follows: (1) removal, when displacing the diaspore further than 5 cm; (2)

226 depulping, when consuming or removing diaspore pulp, with no diaspore removal (<5 cm); (3)
227 interaction, when manipulating diaspore, without depulping or removing the diaspore (<5 cm)
228 (Lima et al. 2013, Costa et al. 2016). We did not considered inspection as an interaction, when
229 ants quickly touched and left the diaspore.

230 *Statistical analyses*

231 We employed generalized linear mixed effects models (GLMMs, *glmer* function for
232 non-normal datasets, with *lme4* package in R) with fixed and random effects to analyze the
233 datasets of diaspore removal experiments (Bolker 2015). In each model, site type (disturbed vs.
234 preserved), treatment (vertebrate-exclosure vs. control) and possible interactions among these
235 factors were considered as predictive variables of fixed effects. Sampling blocks were nested
236 within sites and grouped as random effects to account for the spatial heterogeneity of samples
237 (Bates et al. 2014). The response variables were the proportions of diaspore removed after 48h
238 of exposure in the field, separately for each species. We performed analyses assuming a
239 binomial distribution error of response variable. Regarding the interactions between variables,
240 when significant, we run post-hoc contrast tests (Crawley 2013).

241 To evaluate network structure in disturbed and preserved sites, considering both
242 quantitative and qualitative components of all interaction events which occurred between
243 animals and diaspores piles, we used two network metrics: interactions' Shannon diversity per
244 site (H_2 – see Bersier et al. 2002; Blüthgen et al. 2008) and robustness with regard to cumulative
245 random extinctions of ant species (Memmot et al. 2004; Burgos et al. 2007). In our local
246 networks (i.e., site level), consistent with other studies on ant-plant interactions (e.g., Costa et
247 al. 2018), each interaction frequency was computed based on the interaction between an animal
248 species with an individual diaspore, not the number of workers recruited per diaspore species.
249 Hence, in each site we included all records from all interaction types that occurred between ants

250 and diaspores piles of each of the three species, to build weighted matrices with diaspore species
251 as rows and ant species as columns, filling cells with the number of events observed between
252 one diaspore species *i* and one ant species *j*. Network metrics (i.e. Shannon interaction diversity
253 and robustness) were calculated for each interaction matrix. In total, we had eight
254 matrices/networks corresponding to each site (n=4 per site type). Network metrics were
255 calculated using the R package *bipartite* (Dormann et al. 2008). Thus, each metric value was
256 fitted as response variable, and site type as predictive variable in generalized linear models
257 (GLMs; Crawley 2013). Furthermore, we built a multilayer network comprising all types of
258 events recorded between ants and diaspore species, in order to assess how disturbance might
259 prompt distinct patterns of interaction outcomes. Hence, each network layer corresponded to a
260 distinct type of ant-diaspore association, i.e., removal, depulping and interaction (see Costa et
261 al., 2016 for a similar approach).

262 To access possible differences in seed dispersal effectiveness (SDE) between ant species
263 present in disturbed and preserved sites, we constructed interaction effectiveness landscapes
264 adapted from Schupp et al. (2010, 2017). We combined two variables into an index of ‘diaspore
265 removal effectiveness’ (DRE) for each ant species from disturbed and preserved sites using the
266 formula:

267 Eqn 2: $DRE = QTC \times QLC$.

268 The quantitative component (QTC) corresponded to the frequency of interactions
269 between each ant species and all available diaspores in each site type. The qualitative
270 component (QLC) corresponded to the frequency of diaspore removed by each ant species for
271 all observation bouts in each site type. For each ant species, we computed a measure of total
272 dispersal service that integrates the quality and quantity components of service offered
273 (*effectiveness* package in R). We considered highly-effective dispersers those species that had
274 high values of DRE (high quantitative and qualitative values); inefficient dispersers as those

275 species that had low DRE values (low quantitative and qualitative values); and lowly-effective
276 dispersers as those species that had intermediate DRE values (low quantitative values and high
277 qualitative values or high quantitative values and low qualitative values). We performed all
278 statistical analyses in R (R Core Team 2017) and networks graph using Pajek 4.09 (Batagelj &
279 Mrvar 1998).

280

281 **Results**

282 *Experiment A. Diaspore removal experiment*

283 We found a large variation on diaspore removal among plant species, according to
284 treatments and site types (Table 1). Removal of *Coccoloba* and *Stryphnodendron* diaspores was
285 negligible, smaller than 0.1% across all sites and treatments (Table 1), and thus we excluded
286 these two species from further analyses and will only refer to the other three species from now
287 on. Average diaspore removal rates were 32% for *Byrsonima*, 67% for *Davilla* and, 52% of
288 *Miconia*. However, we found a large range of variation within species (Table 1).

289 We observed higher percentages of diaspore removal in preserved sites compared to
290 disturbed sites for all species (Table 1, Fig. 1). Diaspore removal in the control treatment of all
291 species were 6% to 16% higher than in the vertebrate-exclosure treatment, with highest
292 differences for *Byrsonima* in preserved sites (Table 1). We found higher proportions of
293 diaspores removed in control treatments for the three species, with significant difference for
294 *Davilla* and *Miconia* in both site types, and for *Byrsonima* with significant difference only in
295 preserved areas ($\chi^2=5.16$, $P < 0.1$; Fig. 1). Despite the significant role of vertebrates in diaspore
296 removal, most removal events were attributed to invertebrates in both plot types (Fig. 1).

297 *Experiment B. Diaspore observation experiment*

298 Ants were the only group of animals recorded interacting with the diaspores during the
299 direct diurnal observations. We observed that more than 66% of all diaspore-ant interactions
300 occurred in preserved sites for each of the three plant species monitored (Table 2), with a
301 significant difference between site types for all species (Fig. 2).

302 *Byrsonima* presented the highest number of depulping events (three in each site type), as
303 well as the higher number of diaspores taken into ant nests (two in disturbed sites and one in
304 preserved sites; Table 2). Considering all diaspores removal events for the three plant species
305 in disturbed sites, 24% (i.e. n=4) resulted in the displacement of the diaspore from disturbed to
306 preserved sites. Conversely, no ant species transported diaspores from preserved to disturbed
307 sites (Table 2). The maximum dispersal distance (42 meters) was observed for *Byrsonima*,
308 dispersed by *Atta laevigata* in a preserved site. We found significant differences between
309 diaspore types ($\chi^2= 19.78$, $p=0.043$), with high removal distances for *Byrsonima*, but no
310 significant differences between site types for any of the species (Fig. 3).

311

312 *Experiment B. Ant-diaspore interactions*

313 We recorded 20 ant species performing 477 interaction events with the diaspores, of which
314 65% were recorded in preserved sites (Appendix S3). From all records, 7% represented diaspore
315 removal events and nearly 2% corresponded to diaspore depulping (Table 2). *Crematogaster*
316 sp1, *Pheidole oxyops*, *Pheidole triconstricta* had the highest total number of removal events
317 across all diaspore types and sites (Appendix S3). Three ant species were only registered in
318 disturbed sites: *Brachymyrmex cordemoyi*, *Dolichoderinae* sp. and *Ectatomma tuberculatum*
319 (Appendix S3). *Pheidole triostricla* was the ant species responsible for 46% of diaspore
320 interactions in disturbed sites (Appendix S3). Seed predator ants, such as *Pheidole* spp. and
321 *Dorymyrmex* spp., did not have reduced activities in disturbed sites, with *Pheidole triostricla*

322 even presenting higher number of interactions with diaspores in disturbed sites (Appendix S3).
323 Seven ant species were recorded exclusively in preserved sites: *Brachymyrmex pictus*,
324 *Camponotus rufipes*, *Camponotus* sp1, *Crematogaster* sp1, *Pheidole* sp2, *Pheidole* sp3, and
325 *Pheidole* sp4 (Appendix S3). *Crematogaster* sp1 and *Pheidole triconstricta* were the species
326 with the highest number of interactions with diaspores in preserved sites, representing 22% and
327 21% of all interaction records, respectively (Appendix S3).

328 The DRE landscapes indicated that depending on the site type, different ant species are
329 more effective with regard to the dispersal service provided to plants (Figure 4). In preserved
330 sites, *Pheidole oxyops*, *Camponotus* sp1, *Crematogaster* sp1 and *Pheidole triconstricta* were
331 highly effective dispersers. Conversely, in disturbed sites *Atta laevigata*, *Pheidole oxyops* and
332 *Pheidole triconstricta* were highly effective dispersers. *Pheidole oxyops* and *Atta laevigata*
333 were highly effective in removing diaspores, and presented high values for qualitative
334 components in both site types. *Crematogaster* sp1 and *Pheidole triconstricta* were highly
335 effective in interacting with diaspores in preserved sites, as demonstrated by high quantitative
336 component values for DRE, while in disturbed sites *Pheidole triconstricta*, *Atta laevigata* and
337 *Pheidole oxyops* presented higher quantitative component values for DRE. *Ectatomma*
338 *tuberculatum* presented high values of qualitative component for DRE only in disturbed sites,
339 while *Camponotus* sp1 reached high values of qualitative component in preserved sites. *Atta*
340 *laevigata* achieved distinct DRE values when comparing site types, with an average three times
341 higher in disturbed sites than on preserved sites, as a result of high quantitative component
342 values for DRE on preserved sites. The remaining species that do not appear in the landscape
343 analysis presented very low DRE values.

344 Ant-diaspore interaction networks, formed by three distinct types of interactions
345 (depulping, removing or only interacting) (Fig. 5), were more complex in preserved sites, and

346 presented significant higher values for diversity of interactions ($F=7.91$; $P < 0.05$) and
347 robustness ($F= 6.56$; $P < 0.05$) (i.e., a more stable structure under random and cumulative
348 extinctions of ant species) when compared to networks from disturbed sites (Fig. 6).

349

350 **Discussion**

351 Our data reveals that interactions between ground-foraging animals and diaspores in
352 *campo rupestre* may influence seed fate and contribute to the typical low natural vegetation
353 recovery observed after soil disturbance (Le Stradic et al. 2018b). We found that the quantity
354 and quality of secondary seed dispersal can greatly vary between plant species and
355 environmental conditions, supporting the idea that ground-foraging animals can target specific
356 diaspore species (Roselli 2014), responding in different ways to disturbances (Schowalter et al.
357 1999; Wikars & Schimmel 2001). Our findings indicates that topsoil disturbances modulate
358 secondary seed dispersal by ants, a common interaction in Neotropical savannas (Christianini
359 and Oliveira, 2009, 2010), and other OCBILs, such as the *fynbos* in South Africa and the
360 *kwongan* in Southwestern Australia (Milewski & Bond 1982; Traveset & Rodríguez-Pérez
361 2008). Therefore, our results suggest that topsoil removal reshape the interactions between
362 ground-foraging ants and diaspores, which may result in different outcomes for seed fate and
363 consequently influence the recovery capacity of disturbed old-grow grasslands.

364 Diaspore interactions with ground-foraging animals seems to be reshaped in disturbed
365 soils in *campo rupestre*, intensifying seed limitation and promoting dispersal constraint by: 1)
366 decreasing the proportion and outcome of mutualistic interactions (seed depulping and
367 dispersal); 2) increasing the activity of seed predator ants; 3) reallocating seeds from disturbed
368 to preserved sites; 4) decreasing overall seed dispersal distance. The low diversity and
369 robustness of ant-diaspore networks in disturbed sites suggest its lower stability and more

370 conservative animal-diaspore networks (Bastazini et al. 2019), indicating lower partner
371 diversity (i.e., potential dispersers) and greater vulnerability to collapse in case of ant species
372 loss (or in a wider scale, disperser loss) in disturbed sites. The remarkable differences in ant
373 species composition between sites (35% of the ant species were exclusively found in preserved
374 sites), and the significant differences in diaspore removal effectiveness (DRE) for most ant
375 species between disturbed and preserved sites, reinforces that disturbance can strongly
376 influence secondary diaspore removal dynamics and their ecological outcomes (Schoereder et
377 al. 2004, see also Fernandes et al. 2019). These striking differences in qualitative and
378 quantitative aspects of ant diaspore interactions between sites signalize that “ant limitation”
379 (fewer species, lower abundances) and “dispersal service limitation” can be crucial aspects to
380 better understand plant community assembly and recovery in *campo rupestre*.

381 At least half of diaspores of *Byrsonima*, *Davilla* and *Miconia* were removed within 48
382 hours, indicating that animal-diaspore interactions comprise an important ecological filter
383 driving post-dispersal seed fate for these species and probably for many other plant species in
384 edaphic grasslands. The marked reduction in the removal rates for *Davilla* in disturbed sites
385 indicates the higher vulnerability of the foraging fauna that interact with the seeds of this plant
386 species to soil disturbance. *Byrsonima* presented the higher quantity of depulping events and
387 diaspores-taken-to-ant-nest observed. Likewise, *Byrsonima* diaspores accounted with the
388 higher dispersal distances registered, suggesting a strong role of ground-foraging animals on
389 the diaspore fate for this species and probably congeneric alike species, placing these plant
390 species as a good model for future studies exploring the role of seed-animal interaction
391 outcomes on plant recruitment. The negligible removal rates for *Coccoloba* and
392 *Stryphnodendron* do not mean that their diaspores cannot represent a potential resource for
393 ground-foraging animals, but that in a period of 48 hours these diaspores are unlikely to be
394 removed in *campo rupestre*. Variation in fruit handling by primary avian seed dispersers in

395 *campo rupestre* can be mediate by subsequent interactions among discarded diaspores and
396 ground-dwelling animals, potentially affecting final seed fates (Guerra et al. 2018). Therefore,
397 we should be especially cautious when evaluating the negligible removal rates for *Coccoloba*
398 *cereifera*, which has bird-dispersed berries (Ribeiro & Fernandes 1999).

399 Our study confirms that ant activity as secondary seed dispersers in *campo rupestre*
400 vegetation (Lima et al. 2013, Guerra et al. 2018), is strongly affected by topsoil removal in
401 disturbed areas. Nevertheless, while our diaspore observation experiment was diurnal only,
402 post-dispersal diaspore interactions with vertebrates in *campo rupestre* must not be neglected.
403 In fact, it is sustained by the significant differences found between treatments (vertebrate-
404 enclosure vs. control) in both preserved and disturbed sites, with higher removal rates for all
405 species in the control treatment, meaning that vertebrates do remove between 5 and 16% of
406 diaspores depending on site type and plant species. Guerra et al. (2018) showed that vertebrates
407 can be important as secondary seed dispersers and seed predators in *campo rupestre*. Therefore,
408 these results call attention for the role played by vertebrates as secondary dispersers or seed
409 predators in vegetation recovery and dynamics in *campo rupestre*.

410 The ant species interacting with diaspores encompass well-represented ant genera in
411 *campo rupestre*, such as *Pheidole*, *Camponotus*, *Crematogaster* and *Dorymyrmex* (Costa et al.
412 2015). The differences in the DRE values among ant species suggest specificity in the provision
413 of important ecological services during this critical stage of plant recruitment (e.g. depulping,
414 direct dispersal). Species in *Pheidole* displayed the highest interaction frequency and the
415 highest number of removals, culminating relevant values in DRE in both site types. *Pheidole*
416 and *Atta* are mostly granivorous and the possible outcomes of these interactions in plant
417 community assembly (e.g. seed limitation) for *campo rupestre* should be investigated to better
418 understand its ecological function on seed fate (Christianini et al. 2009; Guerra et al. 2018).
419 Species of *Crematogaster*, *Pheidole* and *Camponotus* presented the highest values for DRE in

420 preserved sites, suggesting their crucial role in structuring the interaction network of diaspores
421 and foraging animals in preserved areas of *campo rupestre*.

422 *Pheidole* spp. seems to be highly tolerant to soil removal disturbance in *campo rupestre*
423 and may hamper natural recovery by seed predation (Denham 2008). *Atta laevigata*, *Pheidole*
424 *triconstricta* and *Pheidole oxyops* were the species with high number of interactions and DRE
425 values in disturbed sites. Also *Atta laevigata* seems to benefit from disturbance (Vieira-Neto
426 et al. 2016), which is supported by a high DRE in disturbed sites, with an average of DRE
427 values three times higher in disturbed than in preserved sites. However, workers of *Atta*
428 *laevigata* may deplete soil seed banks in degraded sites by moving diaspores from degraded to
429 preserved sites, thus limiting plant recruitment (Vaz Ferreira et al. 2011).

430 The predominance of short dispersal distances (< 5 cm) in our experiments are in
431 accordance with previous evidence that show ants as short-distance seed dispersers
432 (Christianini & Oliveira 2010; Gómez & Spadaler 2013; Camargo et al. 2016, Guerra et al.
433 2018). The majority of species observed are small ant species, suggesting that disperser body
434 size is a key trait determining the outcomes of ant-plant interactions (Warren and Giladi 2014;
435 Magalhães et al. 2018). Small ants usually only consume fruit pulps on the spot and do not
436 remove the diaspores far away from their initial location (Ness et al. 2004), which is confirmed
437 by the predominance of high qualitative values of DRE attributed to the larger ant species
438 observed. Our single observation of a long dispersal distance for *Byrsonima* (42 meters) was
439 carried by *Atta laevigata*, the largest ant species observed, reinforcing that large ants are able
440 to provide greater dispersal distances than smaller ones (Ness et al. 2004). Species of the large-
441 body-sized *Ectatomma* dispersed seeds to relevant distances (approximately 10 meters).
442 However, differently from *Atta laevigata*, *Ectatomma* species were responsible for most of the
443 depulping events in preserved sites and were thus a most valuable disperser in preserved sites
444 (Magalhães et al. 2018). Pulp removal is a relevant ecological service, as it decreases the

445 chances of fungal attack (Ohkaware & Akino 2005), and creates conditions for germination of
446 light-demanding seeds, as the case of the *Miconia* (Lima et al. 2013).

447 **Conclusion**

448 Our results suggest that disturbance resulted in structurally different networks involving
449 diaspores and ground-foraging animals, showing that secondary seed dispersal by ants
450 potentially affect vegetation dynamics and resilience. Previous studies have found that variation
451 in secondary diaspore-animal interactions is linked to habitat conservation status (Schupp &
452 Fuentes 1995; Chapman & Chapman 1996; Lambert 2002), and here we show contrasting
453 outcomes arising from animal-plant interactions between disturbed and preserved sites. Topsoil
454 removal affected the robustness of diaspore-animal networks and modified the effectiveness of
455 diaspore removal by ant species, which has potential implications for recruitment.

456 The lack of diaspore dispersal towards disturbed sites and diaspore disposal in preserved
457 sites may strongly influence dispersal limitation and hamper natural recovery (Hopper et al.
458 2016), providing a mechanistic explanation for high vulnerability to soil removal in edaphic
459 grasslands (*sensu* Buisson et al. 2019). Our results become even more relevant considering that
460 *campo rupestre* is a seed-limited ecosystem (Dayrell et al. 2016; Le Stradic et al. 2018), where
461 any reduction in seed quantity may heavily compromises plant recruitment and natural
462 regeneration (Calviño-Cancela 2007; Silveira et al. 2016).

463 In sum, our findings contribute to the current knowledge on establishment filter in a
464 megadiverse tropical grassland, as it provides evidence on how disturbance can reshape
465 diaspore interactions with ground-foraging animals. The next steps should move forward to
466 explore the ecological outcomes on seed fate between preserved and disturbed sites. This will
467 inform how disturbance modulates the establishment filter and support effective conservation

468 and restoration practices (Chambers & MacMahon 1994; Turnbull et al. 2000; Bulot et al.
469 2014).

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479

480 **Authors' contributions**

481 A.J.A, F.A.O.S., T.J.G., F.V.C. and E.B conceived the ideas and designed methodology; A.J.A.,
482 P.A.J., H.T.S.R., T.J.G., F.V.C., J.V.S.M., R.L.C.D and E.B. collected the data; A.J.A., T.J.G.,
483 and F.V.C. analyzed the data; A.J.A. led the writing of the manuscript with inputs from T.J.G.,
484 F.V.C., F.A.O.S and E.B. All authors contributed critically to the drafts and gave final approval
485 for publication.

486

487 **Supporting Information**

488 **Appendix S1.** Photos of the five native diaspores used, three native fruits: *Miconia irwinii*
489 (Melastomataceae), *Byrsonima vacciniifolia* (Malpighiaceae) and *Coccoloba cereifera*

490 (Polygonaceae); and two native seeds: *Stryphnodendron gracile* (Fabaceae) and *Davilla*
491 *elliptica* (Dilleniaceae) (Photos A.J. Arruda).

492 **Appendix S2.** Experiment sampling designs for the 48 hours removal trial and focal
493 experiments.

494 **Appendix S3.** Ant species registered, their functional groups and number of interactions
495 realized according to diaspore and site type for each ant species (PR= preserved, DI=disturbed).

496

497 **Data availability statement**

498 Primary datasets and statistical analysis scripts are available at DOI 10.5281/zenodo.3564943.

499

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781 **Table 1:** Percentage of diaspore removal for five plant species from *campo rupestre* grassland
782 in treatments (vertebrate-exclosure (removal by invertebrates) and control (removal by
783 vertebrates and invertebrates)) in preserved and disturbed sites, at *Serra do Cipó*, southeastern
784 Brazil. Lowercase letters directly above percentage values indicate significant interactions
785 between treatments and sites. When significant, Chi square (χ^2) and P values are presented for
786 the generalized linear mixed model (GLMM) comparing fixed effects and interaction effects.
787 “Site*Treat” represents significant interactions between site and treatments; “Site” represents
788 significant differences between sites; “Treat” represents significant differences between
789 treatments; N.S represents non-significant values for all fixed and interaction effects. See Figure
790 1 for differences between site types and cage treatments for species in bold.

791

Species	Treatment	Preserved	Disturbed	GLMM
<i>Byrsonima vacciniifolia</i>	Exclosure	27% ^a	27% ^a	Site*Treat: $\chi^2=5.16$, p=0.023
	Control	43% ^b	32% ^a	
<i>Davilla elliptica</i>	Exclosure	78%	48%	Site: $\chi^2=31.18$, p<0.001; Treat: X ² =8.11, p=0.004
	Control	83%	58%	
<i>Coccoloba cereifera</i>	Exclosure	<0.1%	<0.1%	N.S.
	Control	<0.1%	<0.1%	
<i>Miconia irwinii</i>	Exclosure	51%	47%	Treat: $\chi^2=17.49$, p<0.001
	Control	57%	57%	
<i>Stryphnodendron gracile</i>	Exclosure	<0.1%	<0.1%	N.S.
	Control	<0.1%	<0.1%	

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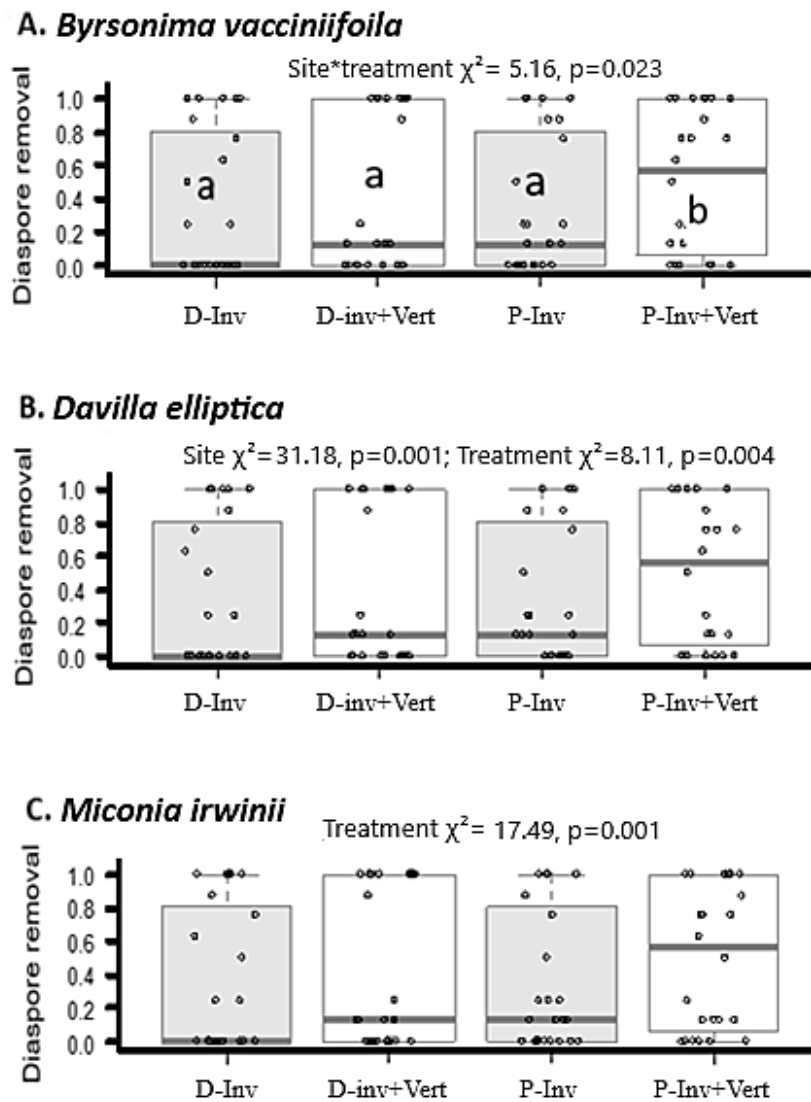
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795 **Table 2:** Number of ant-diaspore interaction types observed for three plant species from
796 disturbed and preserved *campo rupestre* sites, southeastern Brazil. Interaction types were
797 classified as: *Interaction* = total number of animal diaspore associations without pulp or
798 diaspore removal; *Removal*: total number of diaspores removed over more than 5 cm;
799 *Depulping*: total number of events were ants removed diaspores pulp; *Site change* = total
800 number of observations when the final diaspore destination was different from the site of origin;
801 *Ants nest* = total number of diaspores taken into an ant nest.
802
803

Species	Site	Interaction	Removal	Depulping	Site change	Ants nest
<i>Byrsonima vacciniifolia</i>	Disturbed	37	3	3	2	2
	Preserved	110	3	2	0	1
<i>Davilla elliptica</i>	Disturbed	68	6	0	1	1
	Preserved	108	13	1	0	1
<i>Miconia irwinii</i>	Disturbed	60	8	0	1	0
	Preserved	142	4	1	0	0

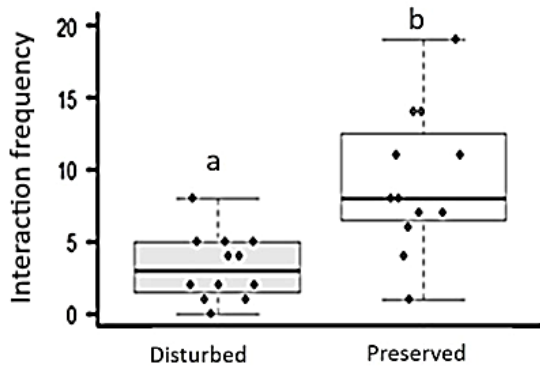
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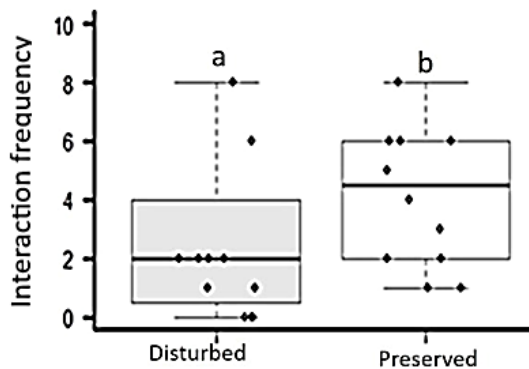
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806 **Figure 1.** Diaspore removal rates in each treatment along disturbed and preserved sites of
 807 *campo rupestre* vegetation, southeastern Brazil. Different lowercase letters inside boxplots
 808 indicate significant statistical differences for *Byrsonima vacciniifolia*, which was the only
 809 diaspore to present significant interactions between treatments and sites (Site*treatment $\chi^2 =$
 810 5.16, $p=0.023$). As no significant interactions between treatments and sites were found for
 811 *Davilla elliptica* and *Miconia irwinii*, only significant statistical values for differences within
 812 treatments and/ or sites are represented above the boxplots: *Davilla elliptica* (Site $\chi^2 = 31.18, p$
 813 $= 0.001$; Treatment $\chi^2 = 8.11, p = 0.004$) and *Miconia irwinii* (Treatment $\chi^2 = 17.49, p = 0.001$).
 814 Inv only = access to invertebrates only = vertebrate exclusion (or wired cage treatment); Vert
 815 + Inv = access to both vertebrates and invertebrates (wired frame control treatment). D =
 816 disturbed sites; P = preserved sites
 817

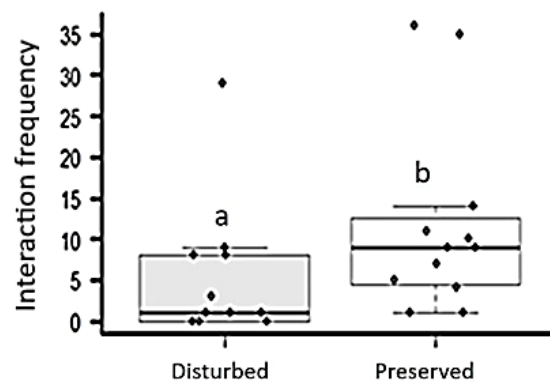
A. *Byrsonima vacciniifolia*



B. *Davilla elliptica*



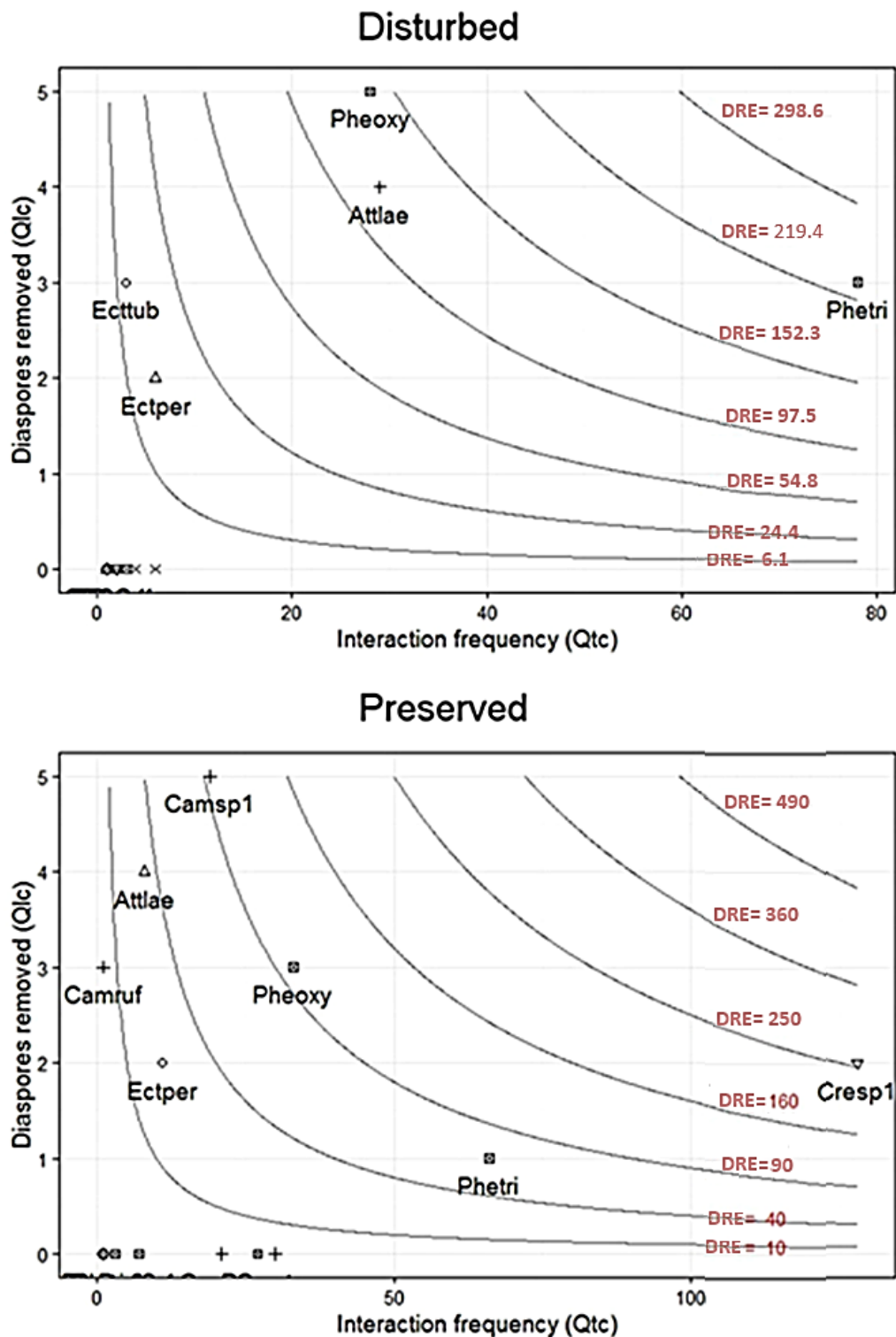
C. *Miconia irwinii*



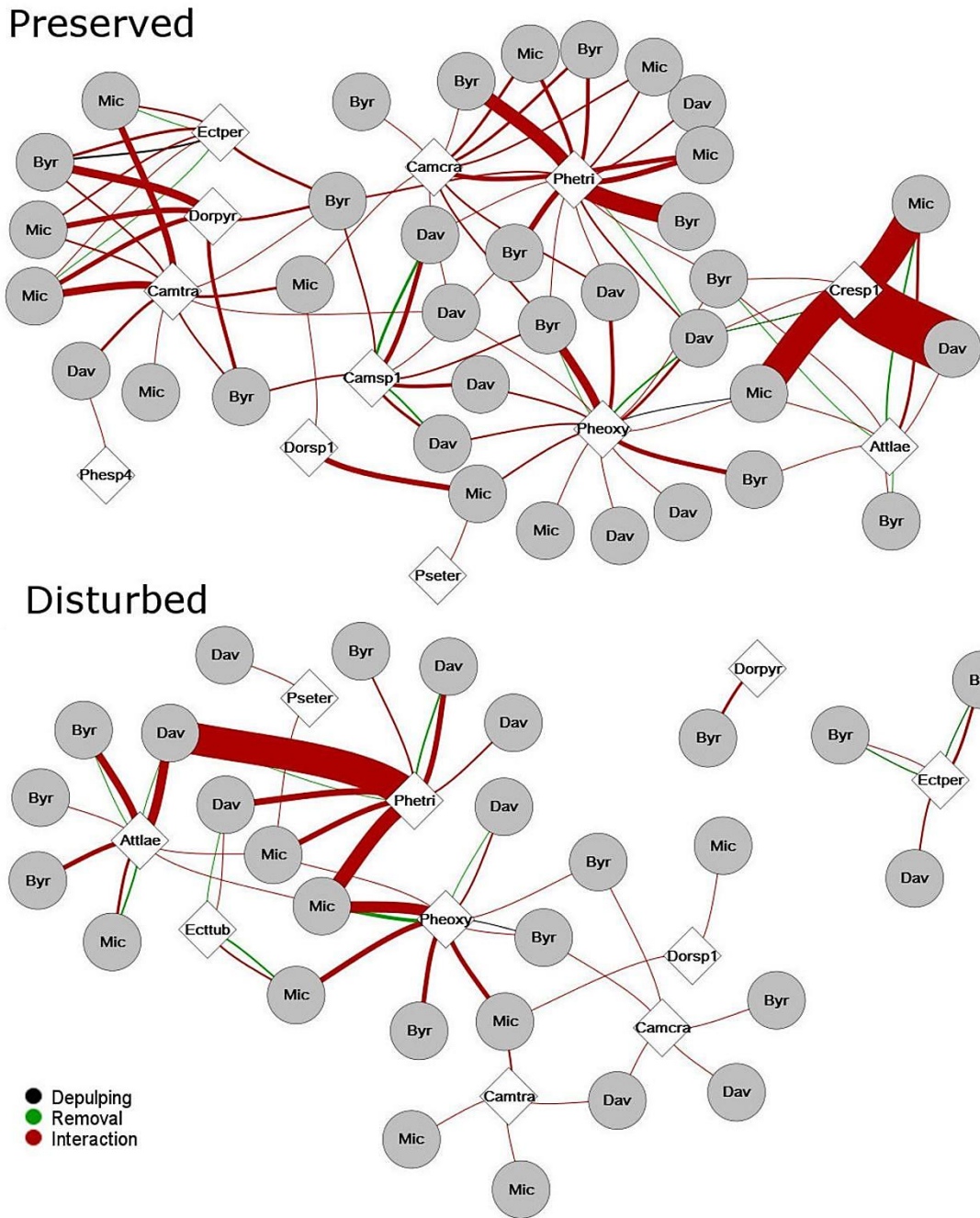
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819 **Figure 2:** Interactions frequency at network level according to site type for three plant species
820 from of *campo rupestre* vegetation, southeastern Brazil. Different letters indicate significant
821 differences between site types for *Byrsonima vacciniifolia* ($\chi^2= 19.78$, $p<0.001$); *Davilla*
822 *elliptica* ($\chi^2=9.17$, $p<0.001$) and *Miconia irwinii* ($\chi^2=19.78$, $p<0.01$). The boxplots represent the
823 median (middle line), 25% and 75% percentiles (the lower and upper boundaries of the boxes,
824 respectively), and the 1.5 interquartile range (whiskers). Black circles indicate individual data
825 points.

826



835
 836 **Figure 4:** Diaspore removal effectiveness landscape (DRE = Quantity × Quality) of all ant
 837 species interacting with diaspores of *Byrsonima vacciniifolia*, *Davilla elliptica* and
 838 *Miconia irwinii* in preserved and disturbed *campo rupestre* sites in southeastern Brazil.
 839 Isoclines represent all combinations of quantity and quality components with the same
 840 value of DRE adapted from Schupp et al. (2017). Symbols represent distinct functional
 841 groups of ant species. Ants' codes and symbols definition can be found in Appendix
 842 S3.

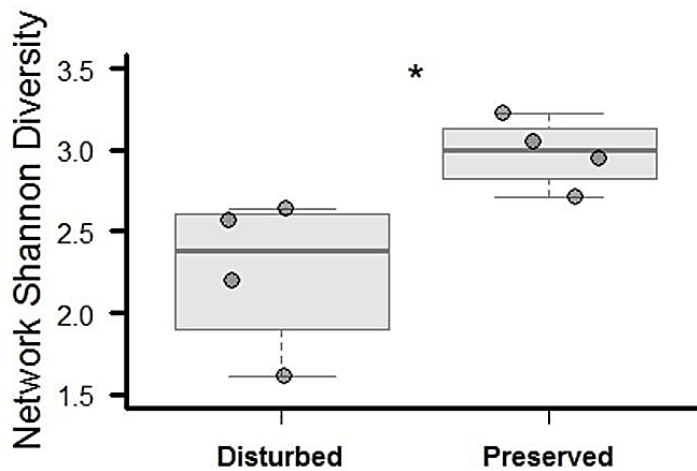


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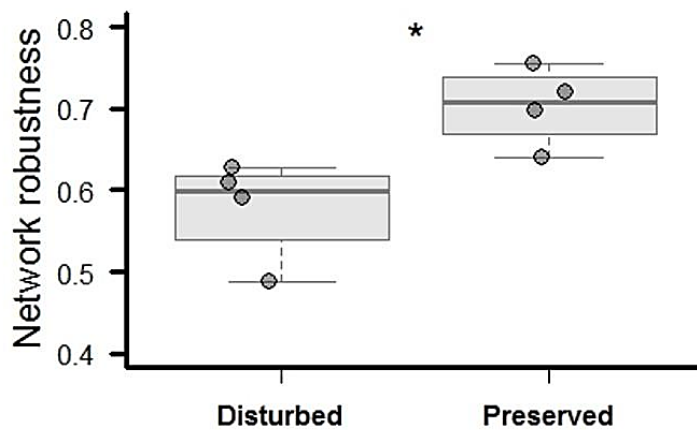
845 **Figure 5:** Ant-diaspore multilayer networks in preserved and disturbed *campo rupestre* sites,
 846 considering distinct types of interactions according to ant behavior (represented by distinct
 847 colors). Line width represents the frequency of interactions. Diamonds depict ant species, with
 848 ant codes provided in Appendix S3. Circles represent distinct stations inside each site.
 849 (*Interaction* = diaspore manipulation without pulp removal or removal further than 5 cm,
 850 *Depulping* = diaspore pulp removal; *Removal* = diaspore displacement further than 5 cm; Byr
 851 = *Byrsonima vacciniifolia*; Dav = *Davilla elliptica*; Mic = *Miconia irwinii*).

852

A) Shannon diversity



B) Network robustness



853

854 **Figure 6:** A) Interactions' Shannon diversity index and B) networks robustness under ant
855 species cumulative random extinctions for ant-diaspores networks in preserved and disturbed
856 *campo rupestre* sites, southeastern Brazil. * represents significant differences between site
857 types for Shannon diversity of interactions ($F=7.91$; $P < 0.05$) and networks robustness ($F=$
858 6.56 ; $P < 0.05$). The boxplots represent the median (middle line), 25% and 75% percentiles (the
859 lower and upper boundaries of the boxes, respectively), and the 1.5 interquartile range
860 (whiskers). Dark gray circles indicate individual data points.