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**Running title:** Disturbance reshapes diaspora interactions

# **Topsoil disturbance reshapes diaspora interactions with ground-foraging animals in a megadiverse grassland**

Andre J. Arruda<sup>1,2,3\*</sup>, Fernanda V. Costa<sup>4</sup>, Tadeu J. Guerra<sup>2</sup>, Patrícia A. Junqueira<sup>2</sup>, Roberta L.C. Dayrell<sup>1,2</sup>, João V. S. Messeder<sup>2</sup>, Hanna T. S. Rodrigues<sup>2</sup>, Elise Buisson<sup>3</sup>, Fernando A. O. Silveira<sup>2,5</sup>

1 University of Western Australia, School of Biological Sciences, Australia

2 Federal University of Minas Gerais, Department of Botany, Brazil

3 Avignon Université, Institut Méditerranéen de Biodiversité et d'Ecologie, CNRS, IRD, Aix Marseille Université, IUT d'Avignon, AGROPARC, France

4 Federal University of Ouro Preto, Graduate School in Ecology of Tropical Biomes, Brazil

5 Federal University of Minas Gerais, Department of Genetics, Ecology and Evolution, Brazil

\*Corresponding author: ajarruda@gmail.com

## Abstract

### *Questions*

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

### *Location*

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

### *Methods*

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

### *Results*

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

### *Conclusions*

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

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

## Introduction

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

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

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

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

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

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

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

## **Material and methods**

### *Study area*

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

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

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

#### *Diaspores from native species*

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

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

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

#### *Removal experiment design*

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



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

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

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

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

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

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

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

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

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

### *Statistical analyses*

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

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

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

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

$$\text{Eqn 2: DRE} = \text{QTC} \times \text{QLC}.$$

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

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

## Results

### *Experiment A. Diaspore removal experiment*

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

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

## Experiment B. Diaspore observation experiment

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

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

## Experiment B. Ant-diaspore interactions

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

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

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

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

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

## Discussion

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

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



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

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

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

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

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

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

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

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

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

## Conclusion

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

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

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

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

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## Authors' contributions

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., 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., and F.V.C. analyzed the data; A.J.A. led the writing of the manuscript with inputs from T.J.G., F.V.C., F.A.O.S and E.B. All authors contributed critically to the drafts and gave final approval for publication.

## Supporting Information

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

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

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

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

#### **Data availability statement**

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

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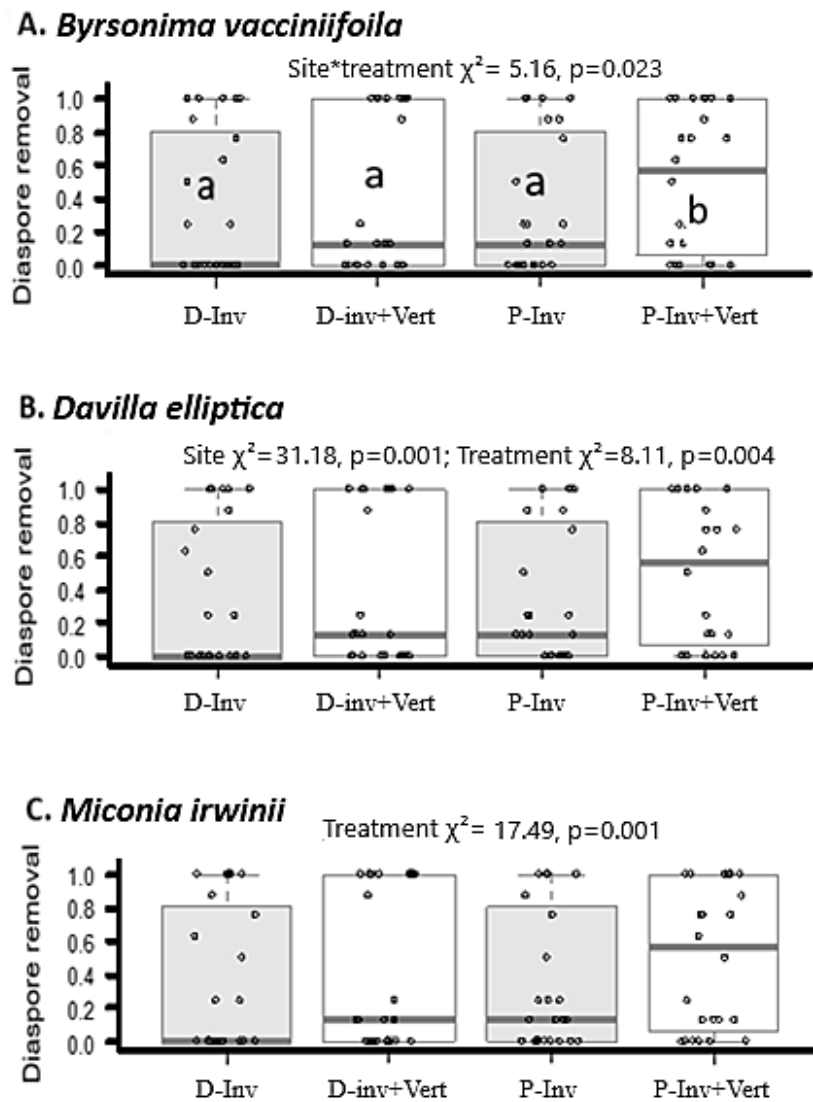
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**Table 1:** Percentage of diaspore removal for five plant species from *campo rupestre* grassland in treatments (vertebrate-exclosure (removal by invertebrates) and control (removal by vertebrates and invertebrates)) in preserved and disturbed sites,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

Species	Treatment	Preserved	Disturbed	GLMM
<b><i>Byrsonima vacciniifolia</i></b>	Exclosure	27% <sup>a</sup>	27% <sup>a</sup>	Site*Treat: $\chi^2=5.16$ , p=0.023
	Control	43% <sup>b</sup>	32% <sup>a</sup>	
<b><i>Davilla elliptica</i></b>	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%	
<b><i>Miconia irwinii</i></b>	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%	

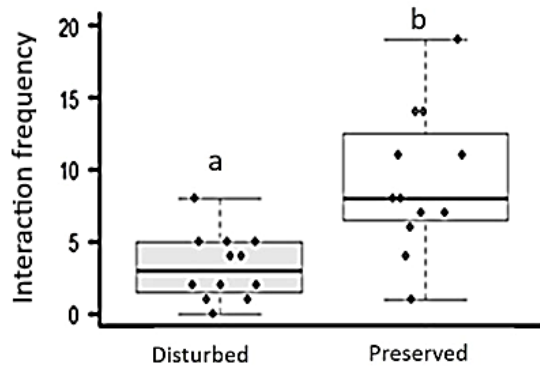
**Table 2:** Number of ant-diaspore interaction types observed for three plant species from disturbed and preserved *campo rupestre* sites, southeastern Brazil. Interaction types were classified as: *Interaction* = total number of animal diaspore associations without pulp or diaspore removal; *Removal*: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

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

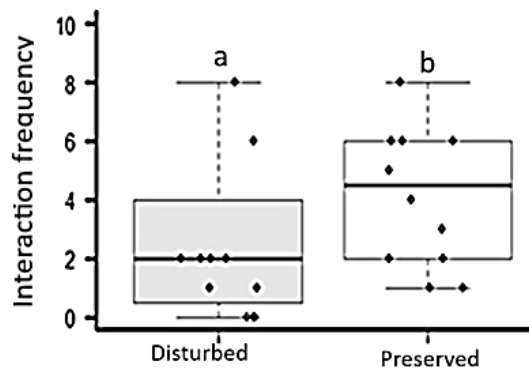


**Figure 1.** Diaspore removal rates in each treatment along disturbed and preserved sites of *campo rupestre* vegetation, southeastern Brazil. Different lowercase letters inside boxplots indicate significant statistical differences for *Byrsonima vacciniifolia*, which was the only diaspore to present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

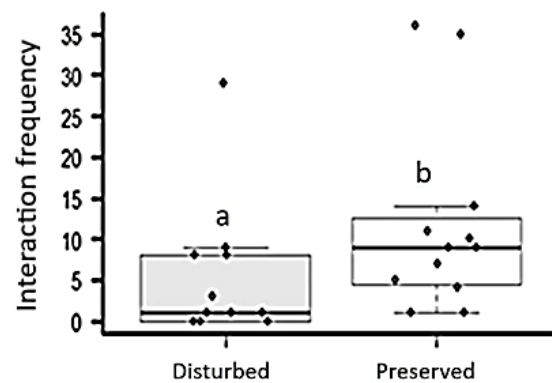
**A. *Byrsonima vacciniifolia***



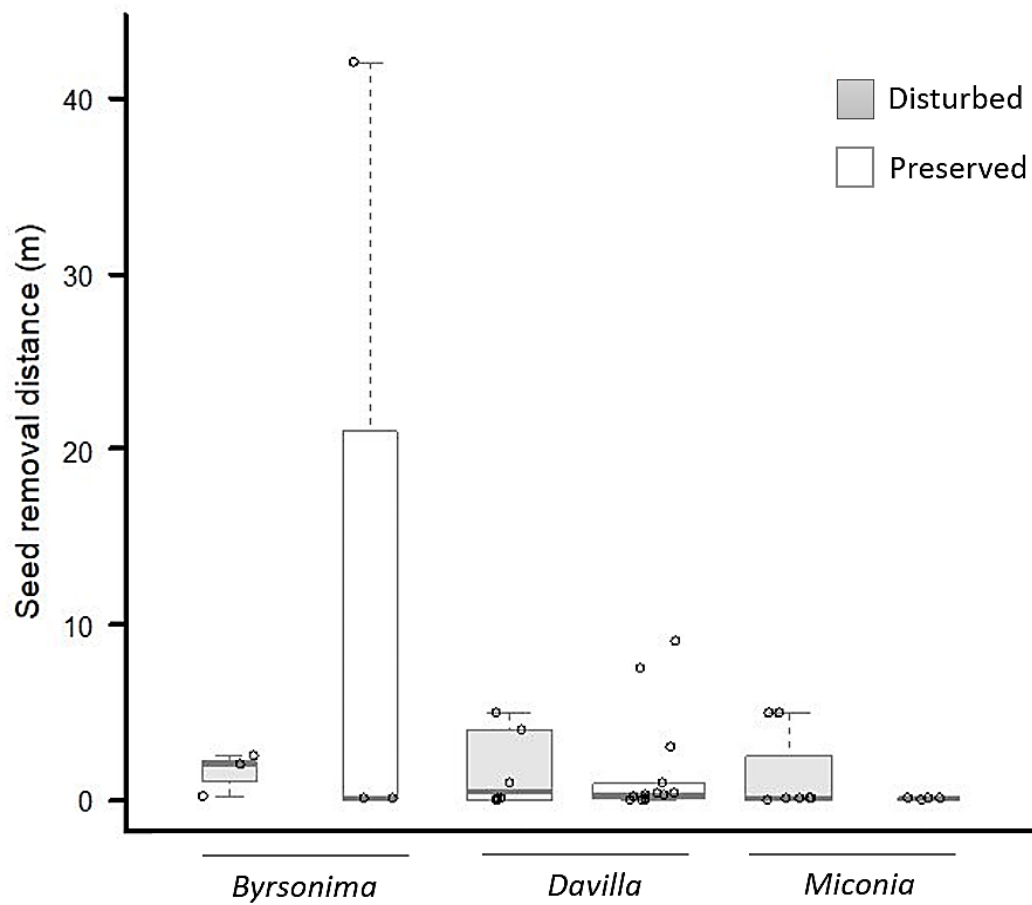
**B. *Davilla elliptica***



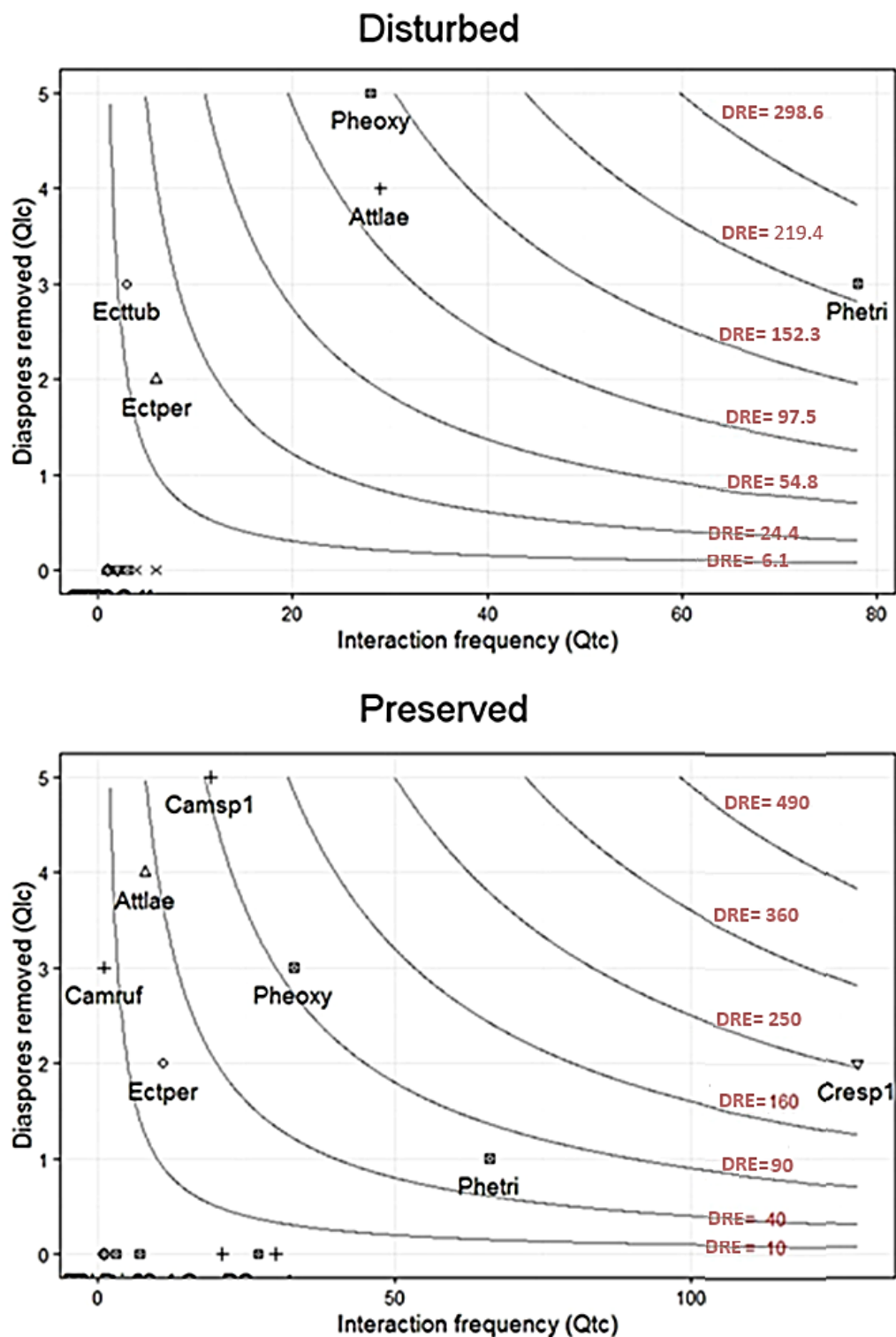
**C. *Miconia irwinii***



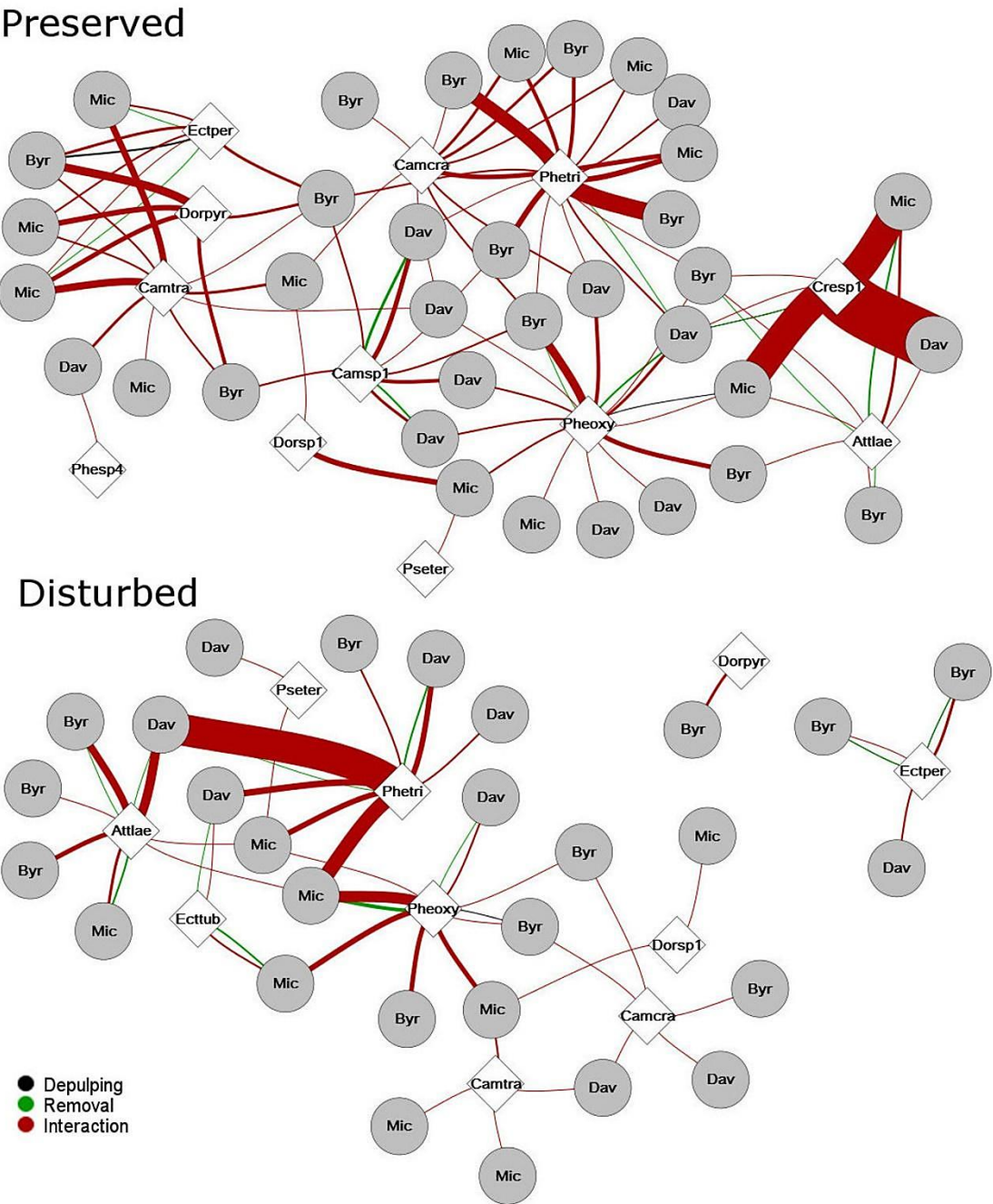
**Figure 2:** Interactions frequency at network level according to site type for three plant species from of *campo rupestre* vegetation, southeastern Brazil. Different letters indicate significant differences between site types for *Byrsonima vacciniifolia* ( $\chi^2= 19.78$ ,  $p<0.001$ ); *Davilla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



**Figure 3:** Mean dispersal distances (meters) for diaspores of *Byrsonima vacciniifolia*, *Davilla elliptica* and *Miconia irwinii* in disturbed and preserved *campo rupestre* sites, southeastern Brazil. The boxplots represent the median (middle line), 25% and 75% percentiles (the lower and upper boundaries of the boxes, respectively), and the 1.5 interquartile range (whiskers). Circles indicate individual data points. Significant differences between diaspores represented by  $\chi^2= 19.78$ ,  $p=0.043$ .



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



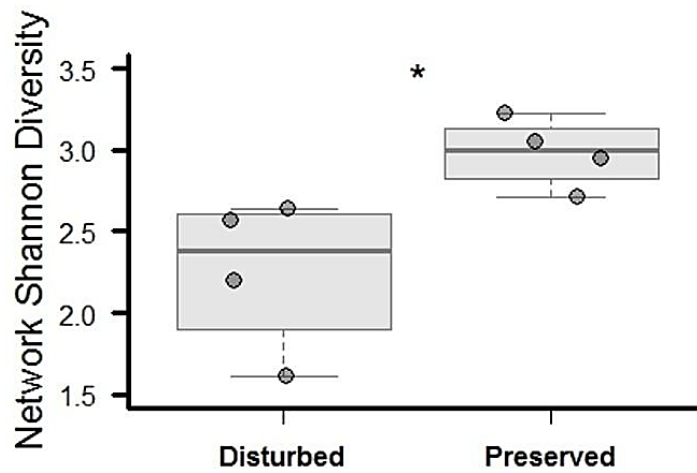
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845 **Figure 5:** Ant-diaspore multilayer networks in preserved and disturbed *campo rupestre* sites,  
846 considering distinct types of interactions according to ant behavior (represented by distinct  
847 colors). Line width represents the frequency 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*).

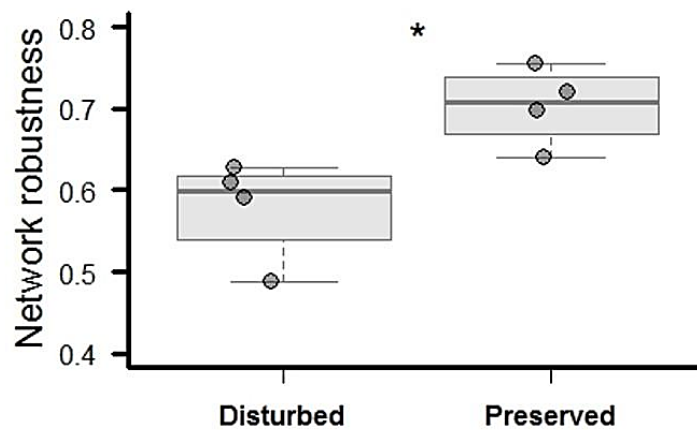
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### A) Shannon diversity



### 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.