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

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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, has been 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 (top soil removal) and preserved sites; we also controlled invertebrate and vertebrate access to determine their role. In addition, we assessed differences in the diaspore removal effectiveness (DRE) and the structure of animal–diaspore interactions through network based-approach.

Results: For three species, the average diaspore removal rate was relatively high (between 30% and 70%). 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 diaspore 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 was more diverse and robust in preserved sites compared with disturbed ones. Seed predator ants (Pheidole and Dorymyrmex) were more common in disturbed sites. Furthermore, significant differences were found 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, impeding 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 impacting upon vegetation dynamics and resilience.

**KEYWORDS**
- ants, *campo rupestrae*, degradation, diaspore removal, myrmecochory, natural recovery, seed limitation, seed predation

1 | INTRODUCTION

Ground-foraging animals may affect seed fate by interacting with diaspores handled or dispersed by primary dispersers (Chambers & MacMahon, 1994; Roberts & Heithaus, 1986; Vander Wall, Kuhn, & Beck, 2005). Empirical studies examining secondary diaspore–animal interactions have shown a strong variability among plant species and habitat types (Chapman & Chapman, 1996; Lambert, 2002; Roselli, 2014; Schupp & Fuentes, 1995), and have been crucial to the development of theoretical models regarding seed fate (Hammond & Brown, 1998; Janzen, 1970; Martinson & Fagan, 2014; Török, Helm, Kiehl, Buisson, & Valkó, 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 (Arnau, Molowny-Horbasz, Rodrigo, & Retana, 2012; Sternberg et al., 2007). Conversely, seed removal can result in seed loss by predation or deposition in unfavourable 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 (Camargo, Rodrigues, Piratelli, Oliveira, & Christianini, 2019; Vander Wall & Longland, 2004). Secondary seed dispersal has been commonly attributed to vertebrates, such as rodents (Feer & Forget, 2002; Genrich, Mello, Silveira, Bronstein, & Paglia, 2017; Lessa, Geise, & Costa, 2013). However, the contribution of invertebrates as secondary diaspore dispersers still remains poorly understood (Camargo et al., 2019; Magalhães, Espírito Santo, Salles, Soares, & Oliveira, 2018). Ants are well-known as seed predators (Costa, Vasconcelos, & Bruna, 2017; Retana, Pico, & Rodrigo, 2004), 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 (Christianini, Mayhê-Nunes, & Oliveira, 2007; Passos & Oliveira, 2003). Even though most diaspores in Neotropical savannas do not present any apparent characteristics that promote ant dispersal (Christianini & Oliveira, 2010), evidence suggests ants to be important seed dispersers of non-myrmecochorous plant species (Christianini et al., 2007; Christianini & Oliveira, 2009, 2010; Guerra et al., 2018; Leal & Oliveira, 1998; Lima, Oliveira, & Silveira, 2013).

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

In old-growth grasslands, plant species disperse poorly and at low rates, making community reassembly a lengthy process (Bond & Parr, 2010; Buisson et al., 2019; Parr, Lehmann, Bond, Hoffmann, & Andersen, 2014; Veldman et al., 2015). In seed-limited ecosystems, such as *campo rupestrae*, 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 rupestrae* (Guerra, Carstensen, Morellato, Silveira, & Costa, 2016; Guerra et al., 2017, 2018; Guerra & Pizo, 2014; Lima et al., 2013), 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 rupestrae*. 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. We then qualitatively compared diurnal animal–diaspore interactions between disturbed and preserved sites for diasprors 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.
2 | MATERIALS AND METHODS

2.1 | Study area

We conducted this study in the vicinity of the Serra do Cipó National Park, in the southern portion of the Espinhaço Range, south-eastern 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 (Bruto 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 (Mucina, 2018; Silveira et al., 2016; Veldman et al., 2015). The landscape encompasses a mosaic formed by patches of rocky outcrops and boulders where sclerophyllous trees and shrubs grow and patches of an herbaceous stratum, which are dominated by monocots and sparsely distributed shrubs (Guerra et al., 2017; Le Stradic, Buisson, & Fernandes, 2015).

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. As a result of this disturbance, the community composition between preserved and disturbed sites still remains drastically different 15 years after the disturbance (Le Stradic, Fernandes, et al., 2018). For the present study, we selected four disturbed sites of at least 100 m² and a preserved (control) site nearby each disturbed one. In all sites, the paired disturbed and preserved sites were adjacent at ~30 m away from each other and each pair were at least 2 km distant from each 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.

2.2 | Diaspores from native species

We used diaspores from five native plant species commonly found in the study area, which have a similar likelihood of being dispersed to disturbed and adjacent preserved sites, to compare diapores removal and interactions with foraging animals between diaporse types and site types (Appendix S1). We selected the species based on four criteria: (a) diaporse size; not too small to prevent the diaspores from being blown away, (b) presence of fleshy parts or rich endosperm to potentially attract both vertebrates and invertebrates, (c) diaporse availability; to allow setting replicates for the removal experiments (between 600 and 1,000 diaspores per species), and (d) phylogenetic and morphological diversity. In our experiment, we used seeds of Stryphnodendron gracile (Fabaceae) and Davilla elliptica (Dilleniaceae). We also 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 is 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 & Francenc, 2015), which typically produces water- and sugar-rich yellow fruits with a striking aroma (Leal, Wirth, & Tabarelli, 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 places them in the refrigerator (4°C) until the commencement of each experiment.

2.3 | Removal experiment design

2.3.1 | Experiment A

We performed randomized block factorial experiments to compare diaporse removal rates between disturbed and preserved sites, and between vertebrate-exlosure 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 × 17 × 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 the presence of wire on diaporse removal (Guerra et al., 2018). We performed five experiments separately, one for each species, simultaneously in all sites for each species. Our removal stations were paired 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, goving a total of 96 samples per plant species (experimental design shown in Appendix S2). Blocks were placed approximately 1 m distant from each other.

The experiment was conducted in the summer from December 2016 to March 2017, which is the period of higher activity by ground-foraging animals (Costa et al., 2018; Pol, Lopez de Casenave, & Pirk, 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 owing to abiotic factors. Using the filter paper was important to gather and count diaspore during experiments. We used white filter papers considering this a less contrasting colour with the white sandy soil in the study sites. Any possible contrast effects by the filter paper leading to attraction or deterrence of animals are present in all treatments.

We placed 10 diaspores per sampling station, giving a total of 960 diaspores per species, with the exception of *Byrsonima* and *Davilla*, in which we placed eight and six diaspores per sampling station, giving 768 and 576 diaspores, respectively. The proportion of diaspores removed in all treatments was evaluated by exhaustively searching for diaspores in stations after 48 h of exposure in the field (Guerra et al., 2018). The proportion of diaspores removed (PDR) from each sampling station was calculated as:

\[
PDR = \frac{N_c \text{ or } N_{re}}{N_o}
\]

where \(N_o\) is the number of offered diaspores, and \(N_c\) and \(N_{re}\) are the number of recovered diaspores in the control treatment and in the vertebrate-exclosure treatment, respectively.

### 2.3.2 Experiment B

To determine the identity of animal species interacting with diaspores, the frequency of interactions, and their behaviour towards the diaspores, we performed direct diurnal observations on diaspore piles in disturbed and preserved sites. To do that, we used diaspores 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 hr 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. The observations were performed during the daytime, always between 10:00 a.m. and 05:00 p.m. Each session comprised 50 min 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 behaviour 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). The specimens collected were also compared with a reference collection from the Insect Ecology Laboratory at UFMG (Costa et al., 2016), in order to ensure species identification. Ant behaviour towards diaspores was classified as: (a) removal, when displacing the diaspore further than 5 cm; (b) depulping, when consuming or removing diaspore pulp, with no diaspore removal (<5 cm); (c) interaction, when manipulating diaspore, without depulping or removing the diaspore (<5 cm; Costa et al., 2016; Lima et al., 2013). We did not considered inspection as an interaction, when ants quickly touched and left the diaspore.

### 2.4 Statistical analyses

We employed generalized linear mixed-effects models (GLMMs, *glmfit* function for non-normal datasets, with *lme4* package in R) with fixed and random effects to analyse 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, Machler, Bolker, & Walker, 2014). The response variables were the proportions of diaspore removed after 48 hr 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 ran 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 (H₂ – see Bersier, Banasek-Richter, & Cattin, 2002; Blüthgen, Fründ, Vázquez, & Menzel, 2008) and robustness with regard to cumulative random extinctions of ant species (Burgos et al., 2007; Memmott, Waser, & Price, 2004). In our local networks (i.e. site level), consistent with other studies on ant–plant interactions (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 diaspore 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, Gruber, & Fründ, 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, Jordano, and Gómez (2010), Schupp, Jordano, and Gómez (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:

\[
\text{DRE} = \text{QTC} \times \text{QLC}
\]

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 low 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).

3 | RESULTS

3.1 | 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 so these two species were excluded from further analyses and henceforth we will only refer to the other three species. Average diaspore removal rates were 32% for Byrsonima, 67% for Davilla and 52% for Miconia. However, a large range of variation was found within species (Table 1).

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

3.2 | 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 65% 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 (Figure 2).

Byrsonima presented the highest number of depulping events (three in each site type), as well as the highest number of diaspores taken into ant nests (two in disturbed sites and one in preserved sites; Table 2). Considering all diaspore 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

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

Notes: Lower case 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, significant interactions between site and treatments; Site, significant differences between sites; Treat, represents significant differences between treatments; N.S., 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 transported diaspores from preserved to disturbed sites (Table 2). The maximum dispersal distance (42 m) was observed for *Byrsonima*, dispersed by *Atta laevigata* in a preserved site. We found significant differences between diaspore types ($\chi^2 = 19.78, p = .043$), with high removal distances for *Byrsonima*, but no significant differences between site types for any of the species (Figure 3).

### 3.3 | 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).
**TABLE 2** Number of ant–diaspore interaction types observed for three plant species from disturbed and preserved *campo rupestre* sites, south-eastern Brazil

<table>
<thead>
<tr>
<th>Species</th>
<th>Site</th>
<th>Interaction</th>
<th>Removal</th>
<th>Depulping</th>
<th>Site change</th>
<th>Ant nests</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Byrsonima vacciniifolia</em></td>
<td>Disturbed</td>
<td>37</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Preserved</td>
<td>110</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>Davilla elliptica</em></td>
<td>Disturbed</td>
<td>68</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Preserved</td>
<td>108</td>
<td>13</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>Miconia irwinii</em></td>
<td>Disturbed</td>
<td>60</td>
<td>8</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Preserved</td>
<td>142</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Notes: 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 were ants removed diaspores pulp; Site change, total number of observations when the final diaspore destination was different from the site of origin; Ant nests, total number of diaspores taken into an ant nest.

Crematogaster sp1, *Pheidole oxyops*, *Pheidole tricornstricta* 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 tristricta* 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 tristricta* even presenting an 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 tricornstricta* 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).

![Figure 2](image)

**FIGURE 2** Interactions frequency at network level according to site type for three plant species from *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 box plots 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.
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; Figure 5), were more complex in preserved sites, and presented significant higher values for diversity of interactions ($F = 7.91; p < 0.05$) and robustness ($F = 6.35; p < 0.05$) (i.e. a more stable structure under random and cumulative extinctions of ant species) when compared with networks from disturbed sites (Figure 6).

4 | DISCUSSION

Our data reveal 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, Fernandes, et al., 2018). 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, 2016; Wikars & Schimmel, 2001). Our findings indicate that topsoil disturbances modulate secondary seed dispersal by ants, a common interaction in Neotropical savannas (Christianini & 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 reshapes 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: (a) decreasing the proportion and outcome of mutualistic interactions (seed depulping and dispersal); (b) increasing the activity of seed predator ants; (c) reallocating seeds from disturbed to preserved sites; and (d) decreasing overall seed dispersal distance. The low diversity and robustness of ant–diaspore networks in disturbed sites suggests its lower stability and more conservative animal–diaspore networks (Bastazini, Debastiani, Azambuja, Guimarães, & Pillar, 2019), indicating lower partner diversity (i.e. potential dispersers) and greater vulnerability to collapse in the 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 found exclusively in preserved sites) and the significant differences in DRE for most ant species between disturbed and preserved sites reinforces that disturbance can strongly influence secondary dias- pore removal dynamics and their ecological outcomes (Schoererder, Sobrinho, Ribas, & Campos, 2004, see also Fernandes, Paolucci, Solar, Neves, & Campos, 2019). These striking differences in qualitative and quantitative aspects of ant–diaspore interactions between sites suggest that ’ant limitation’ (fewer species, lower abundances) and ‘dispersal service limitation’ can be crucial aspects to a better understanding of plant community assembly and recovery in campo rupestre.

At least one-third of the diaspores of *Byrsonima, Davilla* and *Miconia* were removed within 48 hr, 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 greater vulnerability of the foraging fauna that interact with the seeds of this plant species.
to soil disturbance. *Byrsonima* showed higher numbers of depulping events and diaspores-taken-to-ant-nest observed. Similarly, *Byrsonima* diaspores also provided higher dispersal distances, suggesting a strong role of ground-foraging animals on the diaspore fate for this species and probably congenerically alike species. This indicates that these plant species may be 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 would suggest that in a period of 48 hr these diaspores are unlikely to be removed in *campo rupestre*. Variation in fruit handling by primary avian seed dispersers in *campo rupestre* can be ameliorated 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

**FIGURE 4** Diaspore removal effectiveness landscape (DRE = Quantity × 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.
negligible removal rates for *Coccoloba cereifera*, which has bird-dispersed berries (Ribeiro & Fernandes, 1999).

Our study confirms that the activity of ants as secondary seed dispersers in campo rupestre vegetation (Guerra et al., 2018; Lima et al., 2013) 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-exclusion 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 for consideration of 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, Mello, Lana, & Neves, 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). *Pheidole* species 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 & Oliveira, 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, Vasconcelos, & Bruna, 2016), which is supported by a high DRE in disturbed sites, with average

**FIGURE 5** Ant-diaspore multilayer networks in preserved and disturbed campo rupestre sites, considering distinct types of interactions according to ant behaviour (represented by distinct colours). Line width represents the frequency of interactions. Diamonds depict ant species, with ant codes provided in Appendix S3. Circles represent distinct stations inside each site. (Interaction = diaspore manipulation without pulp removal or removal further than 5 cm; Depulping = diaspore pulp removal; Removal = diaspore displacement further than 5 cm; Byr = Byrsonima vacciniifolia; Dav = Davilla elliptica; Mic = Miconia irwinii)
However, values three times higher in disturbed than in preserved sites. However, Atta laevigata workers may deplete soil seed banks in degraded sites by moving diaspores from degraded to preserved sites, thus limiting plant recruitment (Vaz Ferreira, Bruna, & Vasconcelos, 2011).

The predominance of short dispersal distances (<5 cm) in our experiments are in accord with evidence showing ants as short-distance seed dispersers (Camargo, Martins, Feitosa, & Christianini, 2016; Christianini & Oliveira, 2010; Gómez & Espadaler, 2013; 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 (Magalhães et al., 2018; Warren & Giladi, 2014). Small ants usually only consume fruit pulps on the spot and do not remove the diaspores far away from their initial location (Ness, Bronstein, Andersen, & Holland, 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 Byronima (42 m) was carried by Atta laevigata, the largest ant species observed, reinforcing the view that 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 (~10 m). However, unlike Atta laevigata, Ectatomma species were responsible for most of the depulping events in preserved sites and were thus a valuable disperser in preserved sites (Magalhães et al., 2018). Pulp removal is a relevant ecological service, as it decreases the chances of fungal attack (Ohkawara & Akino, 2005) and creates conditions for the germination of light-demanding seeds, as in the case of the Miconia (Lima et al., 2013).

5 | 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 affects vegetation dynamics and resilience. Previous studies have found that variation in secondary diaspore–animal interactions is linked to habitat conservation status (Chapman & Chapman, 1996; Lambert, 2002; Schupp & Fuentes, 1995), 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, Arruda, Buisson, & Silveira, 2016; Le Stradic, Hernandez, et al., 2018), where any reduction in seed quantity may heavily compromise plant recruitment and natural regeneration (Calviño-Cancela, 2007; Silveira et al., 2016).

In summary, our findings contribute to the current knowledge on establishment filters in a megadiverse tropical grassland, as they provide evidence on how disturbance can reshape diaspore interactions with ground-foraging animals. The next steps should be 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 (Bulot, Thierry, Renucci, & Provost, 2014; Chambers & MacMahon, 1994; Turnbull, Crawley, & Rees, 2000).

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AUTHORS’ CONTRIBUTIONS

DATA AVAILABILITY STATEMENT
Primary datasets and statistical analysis scripts are available at https://doi.org/10.5281/zenodo.3564943.

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REFERENCES


**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

**Appendix S1.** The five native diaspores used; three native fruits: *Miconia irwinii* (Melastomataceae), *Byrsonima vacciniifolia* (Malpighiaceae) and *Coccoloba cerifera* (Polygonaceae); and two native seeds: *Stryphnodendron gracile* (Fabaceae) and *Davilla elliptica* (Dilleniaceae) (Photographs A.J. Arruda)

**Appendix S2.** Experiment sampling designs for the 48 hr 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)

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