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Structure of ant-diaspore networks and their functional outcomes in a Brazilian Atlantic Forest

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Abstract

Ants are able to interact with fruits and seeds that are not adapted for ant seed dispersal. In Brazil, several studies show interactions of ants with non-myrmecochorous diaspores; however, few of them have studied the structure of ant-fruit networks. The use of the network approach allows visualising multiple interactions between partners and how they are shaped by the community context. Our study aims to investigate ant-fruit networks as well as quantitative and qualitative dispersal components in a fragment of the Brazilian Atlantic Forest. We investigated the structure of interaction networks, diaspore removal rates, diaspore destination and dispersal distance over two years of observation. We constructed three interaction networks: dry season, rainy season and total, with the latter comprising the two formers. The diaspore removal rate, dispersal distance and diaspore destination experiments were performed for the plant species *Miconia calvescens*, *Miconia prasina*, *Psychotria leiocarpa* and *Inga edulis*. We recorded a large number of interactions, with diaspore cleaning being more frequent than removal. Ant-diaspore networks were nested, non-modular and little specialized. *M. calvescens*, *M. prasina* and *I. edulis* showed higher diaspore removal rates. Diaspore removal distances were the same among *M. calvescens*, *M. prasina* and *I. edulis*. In *M. calvescens* and *I. edulis*, the main diaspore destination was the ant's nest. Our study shows that diaspore cleaning is the most common behavior in ant-diaspore interactions and there are no differences in the organization of interaction networks over the seasons. These results have implications for the future structure of plant communities, considering that a small part of the diaspores is removed, and that most of them are cleaned, favouring germination at the deposition site.

Introduction

Ants and plants interact in a variety of ways, from parasitism to mutualism (Beattie, 1985), including interactions with diaspores (i.e.: dispersal unit) that can result in seed dispersal (Anjos et al., 2020; Luna et al., 2021). Seed dispersal is a fundamental process for plant fitness because it determines the location in which seeds arrive and whether they will be able to develop and reach future stages in that location (Wenny, 2001). Ants can interact with non-myrmecochorous diaspores (without elaiosomes), in which the pulp and aryl

work as an attraction for them (Rico-Gray & Oliveira, 2007). Several ant species have already been reported to disperse non-myrmecochorous diaspores across the globe (Anjos et al., 2020). Pizo and Oliveira (2000), for instance, observed more than 800 interactions between 56 species of non-myrmecochorous plants and 36 species of ants from monthly samplings in the Atlantic Forest over two years.

The interactions between several ant and plant species can be represented by complex ecological networks at the community level, in which species are represented as nodes, and interactions, as links. The use of the network approach



allows visualizing multiple interactions between partners and how they are shaped by the community context, in addition to joining different research fields (Bascompte, 2007). Most studies on ant-plant networks have focused on the interactions between ants and plants with extrafloral nectaries (EFN), and only 6% have studied ant-seed networks (Del-Claro et al., 2018). Furthermore, the majority of such studies have been performed in a few regions, such as the Amazon and Neotropical Savanna, both in Brazil, and on the coast of the Gulf of Mexico (Del-Claro et al., 2018). In interactions between ants and EFN-bearing plants, studies show nested networks (Del-Claro et al., 2018), in which interactions are organised around a central core, and the interactions of less central species are a sub-set of the most generalist species (Bascompte et al., 2003). Studies on mutualistic and predation networks between diaspores and ants also have networks with a nested pattern (Guimar es et al., 2007; Anjos et al., 2018, 2019; Luna et al., 2018), showing that species do not interact randomly. In addition, Anjos et al. (2018) showed that removal and consumption networks are not modular in the Brazilian savanna. Network specialization, vulnerability and robustness were network metrics that were not affected by habitat and exclusion of the main disperser under study in the Mediterranean landscape (Tim teo et al., 2016). Several factors have been pointed out to explain the origin and maintenance of structural patterns of ant-plant networks, such as temperature and precipitation (Rico-Gray et al., 2012), soil characteristics (D ttilo et al., 2013b) and plant phenology (Lange et al., 2013; Anjos et al., 2018). Regarding the latter, as fruiting is seasonal, dry and rainy periods show differences in fruiting plant species, and this can affect the organization of interaction networks.

In addition to a network approach, in order to understand whether plants gain advantages in interactions with ants – such as escape from areas with a high mortality rate, colonization of new areas, and deposition in suitable areas for development (Wenny, 2001; Rico-Gray et al., 2007) –, approaches at the plant-population level are necessary. These approaches allow learning about the effective dispersers, which maximize the number of new adult plants by their dispersal activity, considering the quantitative and qualitative components (Schupp et al., 2010). The number of seeds dispersed represents the quantitative component, and the distance and removal destination represent the qualitative component. Diaspore removal rates by ants – quantitative component – may differ among different plant species, due to diaspore mass and chemical content (Pizo & Oliveira, 2000; Pizo & Oliveira, 2001). The lipid content of diaspores plays an important role in attracting ants for interaction. Small, lipid-rich diaspores (>60%) are highly attended by ants, quickly removed and moved over long distances (>10m) (Rico-Gray et al., 2007). However, ants can also consume diaspore resources locally or remove parts and take them to the nest, which promotes diaspore cleaning (Christianini et al., 2007; Christianini & Oliveira, 2010; Gallegos et al., 2014).

Diaspore cleaning ensures germination rates approximately 20 to 60% higher than control tests (Leal & Oliveira, 1998; Pizo & Oliveira, 1998; Silva et al., 2019), but not for all plant species (Christianini et al., 2007). In addition, diaspore cleaning can increase the germination speed of some species (Pizo & Oliveira, 1998; Silva et al., 2019). Therefore, even without removal, diaspores have an advantage in interacting with ants.

Apart from the number of diaspores removed, the destination and removal distance are important to ensure that seeds are reaching places with improved conditions for seedling establishment and growth. Thus, destination and dispersal distance are part of the qualitative dispersal component (Schupp et al., 2010). The destination of ant-removed diaspores is frequently the ants' nest (Rico-Gray et al., 2007), where they are cleaned and deposited in ant dumps, which are located in subterranean chambers or on the surface (Farji-Brener & Medina, 2000; Giladi, 2006; Luna et al., 2018). Outer dumps are potentially important for plants, as the soil around the nest has different edaphic and microclimatic conditions from those in regular soil, as well as higher nutrient content (Farji-Brener & Medina, 2000). These conditions favour germination in ants' nesting soils for some plant species (Passos & Oliveira et al., 2002; Leal et al., 2007). Diaspore removal generally occurs at short distances, with a global mean dispersal distance of 2.39 m for non-myrmecochorous diaspores (Anjos et al., 2020). Removal distance depends on ants' nest density and on diaspore disposition in relation to the nests. Furthermore, rainforest ecosystems have shorter removal distances than do savanna ecosystems (Anjos et al., 2020). Longer distances and the nest as a destination are more advantageous results for plants (Anjos et al., 2020; Ortiz et al., 2021).

Considering the different behaviors of ant species when interacting with diaspores, the aim of our study was to describe the network structure of ant-diaspore interactions and their temporal variation in the Atlantic Forest in south-eastern Brazil, seeking to understand several aspects of ants' role in the interactions. In order to do so, we attempted to answer the following questions: (1) what is the structure of the ant-diaspore network in the study area? And does this network structure vary between dry and rainy seasons? We expected nested and non-modular networks to be observed, since they seem to be a pattern in mutualistic networks and a variation in this structure according to the season, due to the direct and indirect effect via plant phenology; (2) which ant behavior (removal or cleaning) is more common with diaspores in the community? As cleaning is a behavior with lower energy cost and performed by a wide variety of ant species, we expected it to be more common than removal; (3) what are the removal rates, distances and destinations of the diaspores carried by ants? Finally, we used a series of experiments to study various aspects of the ant-diaspore interaction in four species of plants. Our goal was to learn about the food preference of ants that remove diaspores in the study area, thus revealing their diaspore preferences and the role played by ants in the

quantitative and qualitative components of dispersal. We expected to find different removal rates, removal distances and destinations for different plant species.

Methods

Study Area

We carried out the present study in a secondary forest located on Marambaia Island (23° 02' S; 43° 35' W), on a part of the area known as *Restinga de Marambaia* and located within the municipalities of Rio de Janeiro, Itaguaí, and Mangaratiba, in the Sepetiba Bay, south-eastern Brazil. Although it is called an island, the area corresponds to an enlarged portion of land (ca. 6 km) connected to the continent by a narrow sand strip. The northern part of the island faces the Sepetiba Bay and the southern part faces the Atlantic Ocean (Conde et al., 2005).

Marambaia Island shelters different vegetation types. This diversity of vegetation types is related to the geological processes that formed the island and originated soils with different levels of water saturation (Menezes & Araújo, 2005). Among the most common vegetation types are mangrove, *restinga* (coastal shrub land on sandy soils), and the sloped Atlantic Forest (Conde et al., 2005). The area was farmed from the 17th to the 19th centuries and had most of its forest removed, which is now under secondary succession (Goés et al., 2005). The soil has high leaf deposition, slow decomposition and higher nutrient levels on the surface layer (Pereira et al., 2008).

The regional climate is classified as Aw (Tropical Rainy Climate), according to the Köppen system, with average monthly temperatures above 20 °C. The coldest period occurs from June to August (average minimum temperature around 18 °C), and the warmest period occurs from December to March (average maximum temperature around 30 °C; (Mattos, 2005). Rainfall has an annual average above 1,000 mm. The rainy season consists of November to March, when rainfall indices are above 100 mm (Mattos, 2005).

Ant-plant interactions

This study was conducted in a transect with 0.5 km in length, in a secondary rainforest, and the procedures were performed monthly, from January 2012 to December 2013. We established 50 observation stations every 10 m. This distance is sufficient to preserve independence between ant colonies (Byrne & Levey, 1993; Kaspari, 1993). Each observation station consisted of diaspores on filter paper (10 × 10cm) to facilitate ant visualizing. We collected mature diaspores from trees or which just fallen on the ground. We provided diaspores monthly according to their availability and abundance, ranging from one to 10 diaspores of a single plant species per station. The diaspores made available each month, as well as the total number and stations are presented in supplementary material (Table S1). The diaspores were intercalated among

stations in cases of availability of more than one species on the same day. We set up the stations at 8 a.m. and observed the interactions between diaspores and ants from 9 a.m. to 5 p.m., with two-hour intervals. The lack of nocturnal observations limits this experiment's results, because there are species with nocturnal activity, such as *Odontomachus chelifer*, which are known to remove diaspores (Raimundo et al., 2009). Thus, some species may appear less important in the networks when the interactions actually occur at another time. We recorded the date, time, ant behavior (removal or cleaning) and plant species, as well as collected worker ants for identification. We considered the visualization of any ant species in contact with the surface of the diaspore to be an interaction, as long as the ant was not only walking on the diaspore or touching it with its antennae. Ants of the same species found in the same station at different times of the same day were considered to be the same interaction. The ant specimens were deposited in the Costa Lima Entomological Collection, of *Universidade Federal Rural do Rio de Janeiro (UFRRJ)*. The plant exsiccates were included in the herbarium collection of the Botany Department of UFRRJ (herbarium RBR).

Network Analysis

Considering all observed interactions (removal and cleaning), we constructed interaction matrices A , where $a_{ij} = 1$ when ant species i was observed interacting with diaspore species j , and using $a_{ij} = 0$ where there was no interaction. We built three matrices: one for the whole observation time (referred to here as total network); one for only the dry months (April to October), and one for only the rainy months (November to March). We analyzed the connectance, nestedness, modularity and specialization network metrics. Connectance is the proportion of links made by the total number of possible links in the network (Jordano, 1987). Connectance was obtained by the number of observed connections divided by the number of possible connections, calculated by the formula: $C = I/AP$, where C is connectance; I is the number of observed interactions; A is the number of ant species, and P is the number of plant species (Mello et al., 2011). Nestedness evaluates whether species with few interactions tend to interact with highly interactive species (i.e., generalist species) (Bascompte et al., 2003; Almeida-Neto et al., 2007). We estimated the nestedness value by the NODF index in the ANINHADO software (Guimarães-Jr & Guimarães, 2006). NODF values range from 0 (non-nested) to 100 (perfectly nested). We tested nestedness with 1,000 networks generated by the null model II (Bascompte et al., 2003). Such null model assumes that the probability of an interaction to occur is proportional to the observed number of interactions of both ant and plant species (Bascompte et al., 2003).

In addition, we also evaluated modularity. Modular networks are those in which species interact more frequently with a group than with species outside that group. We estimated modularity in the network by the M index in the MODULAR

software (Marquitti et al., 2013), based on a simulated annealing algorithm (Guimer  & Amaral, 2005). The M Index ranges from 0 (no modules) to 1 (totally separated modules). We used 1,000 randomized networks by the null model II with fixed total margins (Bascompte et al., 2003).

Aiming at a more conservative approach (D ttilo et al., 2016), and counting on the availability of more data in the literature for comparison, we used binary data. Also, it was considered that quantitative and binary data, with the latter defining the fundamental niche of species (Fr nd et al., 2016), can answer different questions. However, in order to measure specialization at the network level, we used quantitative data, where each cell in matrix A was filled with the frequency of interaction between the ant species i and the diaspore species j . We used the H_2' index, which ranged from 0 (completely generalist network, total overlap of interactions) to 1 (completely specialized network, without overlap of interactions). This index is robust to changes in sampling effort and network size (Bl thgen et al., 2006). We simulated 1,000 null networks from each network, using Patefield's algorithm (Patefield, 1981) to evaluate the significance of the H_2' index. We estimated 1,000 H_2' values from the null networks, and then we compared if the observed H_2' value differed from those of the null model.

To test the differences between the dry and rainy periods, we calculated the absolute difference of the H_2' index between the dry and rainy networks, and then compared it with the absolute difference of the same metric for the networks generated by the null models. For NODF and M, which are metrics affected by the network size, we used the methodology adopted by Carvalho et al. (2021), where the observed and randomized values were standardized using z-scores. The transformed Z-score is defined: $Z = [x - \mu] / \sigma$, where x is the observed index value, μ is the mean of the values from the null networks, and σ is the standard deviation of the values from the null networks (Almeida-Neto et al., 2008). We used the calculated values of NODF and M for the null networks obtained on ANINHADO and MODULAR, respectively. We estimated the significance of the difference in the metrics between the dry and rainy networks using z-scores with values greater than 2 (Dormann & Strauss, 2014).

Moreover, we defined whether the species were central or peripheral in the networks by the formula: $G_c = (k_i - K_{mean}) / \sigma_k$, where k_i is the mean number of connections for a given ant or diaspore species; K_{mean} is the mean number of connections for all ant or diaspore species (connectance), and σ_k is the standard deviation of the number of connections for ant or diaspore species (D ttilo et al., 2013a). $G_c > 1$ values indicated central species in the network, with a large number of connections. $G_c < 1$ values indicated peripheral species in the network, with few connections. With the exception of nestedness and modularity, all other analyses cited were performed using the 'bipartite' package (Dormann et al., 2019) implemented in R v.4.0.2 (R Development Core Team, 2020).

Diaspore removal rate

We selected four species of plants with high abundance of individuals and a large number of fruits in the study area: *Inga edulis* Mart., *Miconia calvescens* DC., *M. prasina* (Sw.) DC. And *Psychotria leiocarpa* Cham. & Schlttdl. (Table 1). From June to October 2013, we picked 15 stations at random where we placed diaspores protected by screen fences (20 × 20 × 12 cm; 2-cm gap) fixed on the ground by 2-cm wires, so as to allow ant access and prevent disturbance by vertebrates. We then placed four diaspores of a single species in each station. We marked the diaspores with a small dot using a marker pen to identify the fruits utilized in the experiment. We set up the experiment at 07:00 a.m. and then checked it after 24 h, when we recounted the number of diaspores. We considered a diaspore removed if we did not find it in a radius of 30 cm around the cage (Passos & Oliveira, 2002). We repeated the experiment during the fruiting period of each evaluated diaspore species and while there were enough diaspores to perform the experiment. We used GLMM with the Poisson family to test for differences in the number of removals between diaspore species. We considered the number of removals to be the dependent variable; diaspore species, the fixed factor; and stations, a random factor. The analyses were performed using the 'lme4' (Bates et al., 2019) and 'multcomp' (Hothorn et al., 2008) packages implemented in R v.4.0.2 (R Development Core Team, 2020).

Destination and distance of diaspore removal

From June to October 2013, we selected one fruiting individual of each plant species selected in the previous experiment (Table 1), and established three radial stations underneath it at approximately 1 m from each other, in which we assessed the diaspore destination and distance. Each station was composed of filter paper (10 × 12 cm) used as substrate, and received two diaspores. Thus, each observed plant had a total of six diaspores. We monitored the stations for ant removal from 8:00 a.m. to 5:00 p.m. with one-hour pauses between observations, for a total of 5 observation hours per day. Observations were suspended during rain. The total hours observed in each species are shown in Table 1. When a removal event took place, we followed the ants to their nests or until they disappeared in the leaf litter, and then measured the removal distance with a measuring tape. We replaced each diaspore after removal.

We used GLMM with the Binomial family to test for differences in destination between diaspore species. We considered the destination the dependent variable; diaspore species, the fixed factor; and data and time, random factors. To test differences in removal distance between diaspore species, we used GLMM with the Gaussian family. We considered the removal distance the dependent variable; diaspore species, the fixed factor; and data and time, random factors. The analyses were performed using the 'lme4' (Bates et al., 2019) and 'multcomp' (Hothorn et al., 2008) packages implemented in R v.4.0.2 (R Development Core Team, 2020).

Table 1. Plant species used in the removal-rate, destination and removal-distance experiments. Observation hours refer to the total time of removal-distance experiments.

Plant species	Diaspore size (cm)	Observation hours
<i>Inga edulis</i> Mart.	1.20 ± 0.17 × 0.77 ± 0.13	5
<i>Miconia calvescens</i> DC.	0.42 ± 0.05 × 0.37 ± 0.04	25
<i>Miconia prasina</i> (Sw.) DC.	0.51 ± 0.04 × 0.42 ± 0.04	25
<i>Psychotria leiocarpa</i> Cham. & Schltdl.	0.57 ± 0.09 × 0.49 ± 0.06	26.5

Results

Ant-plant interactions

We recorded a total of 1,032 interactions among 49 ant species (22 genera belonging to six subfamilies) (Table 2) and 25 plant species belonging to 17 families (Table 3). Myrmicinae was the subfamily of ants with the largest number of species (S = 36 spp.; 73.47%), followed by Ponerinae (S = 4 spp.;

8.16%) and Formicinae (S = 4 spp.; 8.16%). For plants, the Melastomataceae, Meliaceae and Rubiaceae families equated to over 50% of the interactions.

We observed 1,016 diaspore cleaning interactions (98.45%) versus 16 diaspore removal interactions (1.55%). However, 275 diaspores disappeared from the observation area and may have been removed by the ants. We have included these diaspores as removals in Table 3, which, therefore, has a different total number of removals from that shown here. Considering these disappearances to be removals, the total number of removals was 291 (22.26% of interactions). The inclusion of such data overestimates removal by ants; however, the specific removal experiments (results in the topic below) showed that the removal was greater than 1.55%. The true percentage of diaspore removal by ants in the community must be between these two values (1.55% and 22.26%). Ants that removed diaspores were *Acromyrmex subterraneus*, *Atta sexdens rubropilosa*, *Cyphomyrmex* sp. 1, *Ectatomma edentatum*, *E. permagnum*, *Neoponera apicalis*, *Pachycondyla striata*, *Pheidole sigillata* and *Sericomyrmex* sp. 1.

Table 2. Ant species recorded in this study on Marambaia Island (RJ) interacting with diaspore species.

Sub-family / Species	Code	Sub-family / Species	Code
Dolichoderinae		Myrmicinae (Continuation)	
1. <i>Linepithema</i> sp. 1	Linep1	25. <i>Pheidole</i> sp. 10	Pheid10
Ectatomminae		26. <i>Pheidole puttemansi</i> Forel, 1911	Ph_put
2. <i>Ectatomma edentatum</i> Roger, 1863	E_ede	27. <i>Pheidole lucaris</i> Wilson, 2003	Ph_luc
3. <i>Ectatomma permagnum</i> Forel, 1908	E_per	28. <i>Pheidole</i> sp. 13	Pheid13
Formicinae		29. <i>Pheidole</i> sp. 14	Pheid14
4. <i>Brachymyrmex</i> sp. 1	Brach1	30. <i>Pheidole sensitiva</i> Borgmeier, 1959	Ph_sen
5. <i>Brachymyrmex</i> sp. 2	Brach2	31. <i>Pheidole</i> sp. 16	Pheid16
6. <i>Camponotus</i> sp. 1	Campo1	32. <i>Pheidole</i> sp. 17	Pheid17
7. <i>Myrmelachista</i> sp. 1	Myrme1	33. <i>Sericomyrmex</i> sp. 1	Seric1
Myrmicinae		34. <i>Solenopsis</i> sp. 1	Solen1
8. <i>Acromyrmex subterraneus</i> Forel, 1893	A_sub	35. <i>Solenopsis</i> sp. 2	Solen2
9. <i>Atta sexdens rubropilosa</i> Forel, 1908	A_sex	36. <i>Solenopsis</i> sp. 3	Solen3
10. <i>Carebara urichi</i> (Wheeler, 1922)	C_uri	37. <i>Solenopsis</i> sp. 4	Solen4
11. <i>Carebarella bicolor</i> Emery, 1906	Careb1	38. <i>Solenopsis</i> sp. 5	Solen5
12. <i>Crematogaster</i> sp. 1	Crema1	39. <i>Solenopsis</i> sp. 6	Solen6
13. <i>Cyphomyrmex</i> sp. 1	Cypho1	40. <i>Trachymyrmex</i> sp. 1	Trach1
14. <i>Mycocarpus</i> sp. 1	Mycoc1	41. <i>Trachymyrmex</i> sp. 2	Trach2
15. <i>Octostruma rugifera</i> (Mayr, 1887)	O_rug	42. <i>Wasmannia auropunctata</i> (Roger, 1863)	W_aur
16. <i>Pheidole diligens</i> (Smith, 1858)	Ph_dil	43. <i>Wasmannia</i> sp. 2	Wasm2
17. <i>Pheidole sigillata</i> Wilson, 2003	Ph_sig	Ponerinae	
18. <i>Pheidole</i> sp. 3	Pheid3	44. <i>Odontomachus chelifer</i> (Latreille, 1802)	O_che
19. <i>Pheidole transversostriata</i> Mayr, 1887	Ph_tra	45. <i>Odontomachus meinerti</i> Forel, 1905	O_mei
20. <i>Pheidole pedana</i> Wilson, 2003	Ph_ped	46. <i>Neoponera apicalis</i> (Latreille, 1802)	N_api
21. <i>Pheidole subarmata</i> Mayr, 1884	Ph_sub	47. <i>Pachycondyla striata</i> Fr. Smith, 1858	P_str
22. <i>Pheidole</i> sp. 7	Pheid7	Pseudomyrmecinae	
23. <i>Pheidole tijucana</i> Borgmeier, 1927	Ph_tij	48. <i>Pseudomyrmex</i> sp. 1	Pseud1
24. <i>Pheidole</i> sp. 9	Pheid9	49. <i>Pseudomyrmex</i> sp. 2	Pseud2

Table 3. Diaspore species explored by ants in this study on Marambaia Island (RJ) with the total number of interactions recorded and ant species with which they interacted (see code in Table 2).

Family / Species	Unit of dispersal	Number of interactions	Number of removals	Ant species
Araceae				
50. <i>Monstera adansonii</i> var <i>klotzschiana</i> (Schott) Madison	Fruit	4	1	18-19; 34; 38
Burseraceae				
51. <i>Protium brasiliense</i> Engl.	Seed	61	41	3; 16-21; 25-26; 33-38; 42; 44; 47
Erythroxylaceae				
52. <i>Erythroxylum pulchrum</i> A. St.-Hil.	Fruit	74	19	12; 16-21; 25-27; 29-30; 34-36; 38-42; 47
Fabaceae				
53. <i>Inga edulis</i> Mart.	Seed	23	2	1; 3; 12; 16-19; 25; 40-41; 46-47
Lauraceae				
54. <i>Ocotea schottii</i> (Meisn.) Mez	Fruit	89	1	1-2; 4-5; 14; 16-22; 25; 33-35; 38-40; 42; 48
Malpighiaceae				
55. <i>Niedenzuella acutifolia</i> (Cav.) W.R. Anderson	Fruit	1	1	42
Melastomataceae				
56. <i>Clidemia hirta</i> (L.) D. Don	Fruit	26	17	16-21; 35; 40; 42-43; 47
57. <i>Miconia calvescens</i> DC.	Fruit	70	30	1; 4; 8; 12; 16-21; 23; 25; 34; 38; 42
58. <i>Miconia prasina</i> (Sw.) DC.	Fruit	193	93	1; 3-4; 12; 16-21; 23; 25; 28; 33; 35; 38; 40-41; 43; 46; 49
Meliaceae				
59. <i>Guarea guidonia</i> (L.) Sleumer	Seed	132	35	2-3; 10; 12; 16-27; 34; 36-38; 42-43; 45-47
Moraceae				
60. <i>Ficus insipida</i> Willd.	Fruit	78	0	1; 3; 7; 10; 12; 16-21; 25; 33-38; 40; 42-44; 47
Nyctaginaceae				
61. <i>Guapira opposita</i> (Vell.) Reitz	Fruit	7	0	16; 18-19; 25; 36; 47
Passifloraceae				
62. <i>Passiflora edulis</i> Sims	Seed	18	1	14; 16-20; 35-36; 42
Piperaceae				
63. <i>Piper amplum</i> Kunth	Fruit	2	0	6; 19
64. <i>Piper anisum</i> (Spreng.) Angely	Fruit	3	0	16; 40; 42
65. <i>Piper caldense</i> C. DC.	Fruit	5	0	9; 13; 19; 43
Rubiaceae				
66. <i>Coccocypselum cordifolium</i> Nees & Mart.	Fruit	17	0	16-22; 36; 42
67. <i>Psychotria</i> cf. <i>hoffmannseggiana</i> (Schult.) Müll. Arg.	Fruit	14	1	1; 16-22; 31
68. <i>Psychotria deflexa</i> DC.	Fruit	37	10	11-12; 16-20; 36; 40; 42-43; 47
69. <i>Psychotria leiocarpa</i> Cham. & Schltldl.	Fruit	58	14	16-22; 28; 32; 34; 36; 40-43
Sapindaceae				
70. <i>Paulinia micrantha</i> Cambess.	Seed	33	6	9; 11; 16-21; 25; 33; 36; 40; 42
71. <i>Urvillea</i> sp.	Fruit	3	0	18; 20
Siparunaceae				
72. <i>Siparuna guianensis</i> Aubl.	Seed	17	17	16-19; 29; 42
Solanaceae				
73. <i>Solanum pseudochina</i> Spreng.	Fruit	21	0	10; 16-20; 22; 36; 40; 47
Verbenaceae				
74. <i>Citharexylum myrianthum</i> Cham.	Fruit	46	2	1; 15-21; 25; 34-36; 40; 42-43; 47

The network of only dry months showed a connectance of 0.271; it was significantly nested (NODF = 34.34; $p < 0.001$), not significantly modular ($M = 0.25$; $p = 0.99$), and it had a higher level of specialization than the null models ($H_2' = 0.123$; $p < 0.001$). The ant species in the central core were *Pheidole* species (Species 16-20 – Table 2) and *W. auropunctata*. The diaspores in the central core were *O. schottii* (Lauraceae), *M. prasina* (Melastomataceae) and *G. Guidonia* (Meliaceae). The network of only rainy months showed a connectance of 0.279; it was significantly nested (NODF = 36.03; $p < 0.001$), not significantly modular ($M = 0.25$; $p = 0.99$), and it had no higher level of specialization than the null models ($H_2' = 0.118$; $p = 0.086$). The ant species in the central core were *Pheidole* species (Species 16-21 – Table 2), *Solenopsis* sp. 3, *W. auropunctata* and *P. striata*. The diaspores in the central core were *Protium Guidonia brasiliense* (Burseraceae), *E. pulchrum* (Erythroxylaceae) and *G. guidonia* (Meliaceae).

Diaspore removal rate, destination and distance of diaspore removal

Miconia calvescens, *M. prasina* and *Inga edulis* showed the highest removal rates (65.8%, 58.3% and 40.0%, respectively), whereas *P. leiocarpa* showed a low removal rate (2.1%; deviance = 310.03; d.f = 3; $p < 0.001$, Fig 2a).

In the experiments on destination and distance of diaspore removal, we observed 137 removals for *M. calvescens*, 14 for *M. prasina* and 17 for *I. edulis*. No removals were observed for *P. leiocarpa*. The ants that removed diaspores were *A. sexdens rubropilosa* and poneromorph species (*Pachycondyla* and *Ectatomma* species). The *A. sexdens rubropilosa* species removed the most diaspores of *M. calvescens* (95.62%; $n = 131$), whereas the poneromorph species removed the most diaspores of *M. prasina* (92.86%; $n = 13$). The *A. sexdens rubropilosa* species removed all the diaspores of *I. edulis*. The removal distance varied between 5 and 473 cm. The average removal was 107.50 cm for *M. calvescens*, 104.57 cm for *M. prasina* and 300.55 cm for *I. edulis*. The diaspores of *I. edulis* were removed farther than *Miconia* diaspores (deviance = 1945.3; d.f = 3; $p < 0.001$). Ants carried most of the diaspores of *M. calvescens* and *I. edulis* to their nests (83.94% and 94.12%, respectively; Fig 2b). In most *M. prasina* removals, ants did not reach the nests and abandoned the diaspores in the leaf litter (92.86%; deviance = 134.02; d.f = 2; $p < 0.001$; Fig 2b). We observed pieces of *M. calvescens* fall to the ground along the path of *A. sexdens*. We did not observe any subsequent discards by *A. sexdens* for any plant species after they entered the nest.

Discussion

This study recorded a large number of interactions in an Atlantic Forest area on Marambaia Island, with diaspore cleaning being the main interaction. The networks analysed cleaning and removal interactions together, and

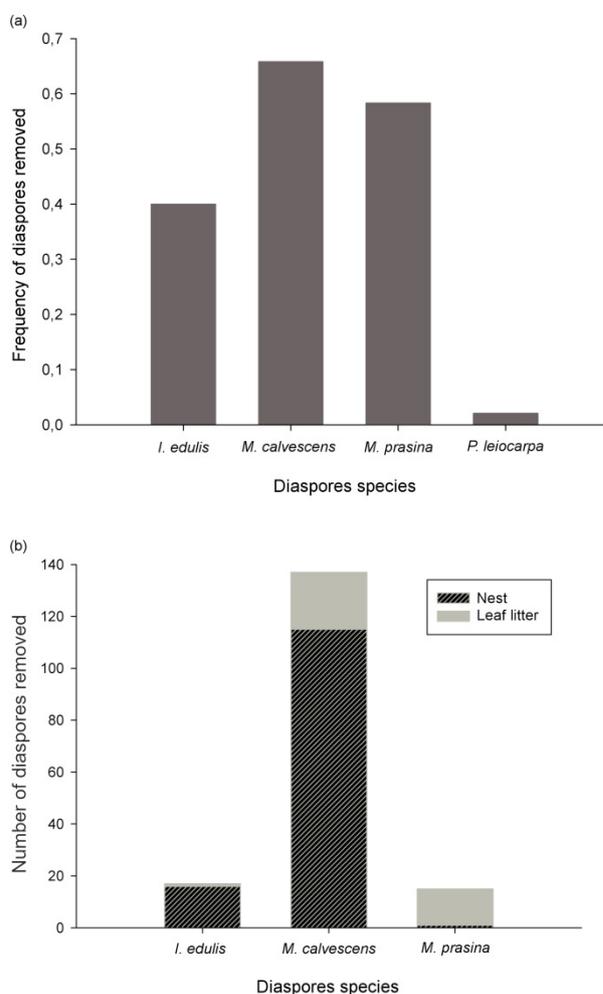


Fig 2. (a) Diaspores removal frequency of the plant species studied. (b) Destination of diaspore removals (nest = black hatched bar, litter = grey bar).

they were nested and without modules for the total, dry and rainy seasons networks. The total and dry season networks were more specialized than the null models, but with low specialization values. The rainy season network did not show higher specialization than the null models. The central core ant species were virtually the same in the dry and rainy seasons. Removal rates were high and equal for the *Miconia* and *Inga edulis* species, but low for *Psychotria leiocarpa*. The removal distance was the same for the *Miconia* species and *I. edulis*. However, the destination of *M. calvescens* and *I. edulis* was mostly the nest, while for *M. prasina* the destination was the leaf litter.

Ant-plant interactions

Diaspore cleaning was the most common ant behavior in the recorded interactions, being 3.5 times more frequent than diaspore removal. This result opposes to that found in ant-fruit interaction networks in the Brazilian *Cerrado*, where diaspore removal was more common than diaspore cleaning (Anjos et al., 2018). Despite not presenting values for comparison, Pizo and Oliveira (2000) report that the behavior

of removing pieces and collecting liquids is more common than removing diaspores in the Atlantic Forest. In addition, Passos and Oliveira (2003) justify their systematic sampling in a *restinga* area by pointing the high speed of diaspore removal by large ponerines, which makes it difficult for them to be seen during active search. Moreover, ants remove diaspores at longer distances in savanna areas than in rainforests (Anjos et al., 2020). In addition to the distance, the removal rate may also be lower in rainforests. Moreover, impacted areas of the Atlantic Forest and in secondary succession had lower removal rates when compared to undisturbed areas (Zwiener et al., 2012; Almeida et al., 2013; Bieber et al., 2014). Poneromorph species are the ant species that most remove in Atlantic Forest areas, and they are less often observed in impacted areas than in undisturbed areas (Almeida et al., 2013; Bieber et al., 2014). The fact that the study area was in the process of secondary succession explains the low removal rate. In addition, most interacting ant species were small and did not remove diaspores. An ant's body size is also a key trait for removal (Camargo et al., 2019).

Diaspore cleaning plays an important role in plant recruitment, since it increases germination rates in most plant species whose diaspores are cleaned by ants (Christianini et al., 2007; Camargo et al., 2016), and it may also decrease germination time (Lima et al., 2013). In addition, diaspore cleaning decreases the chances of attacks by pathogens such as fungi, providing conditions for germination and seedling development (Pizo & Oliveira, 1998; Passos & Oliveira, 2002). For example, *Guarea guidonia*, whose diaspores are cleaned by ants, is benefited by this interaction (Silva et al., 2019). Therefore, although there is no removal, diaspore cleaning brings benefits to the plants.

The *M. prasina* species was the most frequent in the interaction records, followed by *G. guidonia*. *Miconia* species are classified as ornitochoric and usually have high water and sugar content (Silveira et al., 2012). The compounds present in these fruits serve as a resource for the ants and should promote the high number of interactions found for that species. *Guarea guidonia* is an ornitochoric species whose seeds are covered with a red sarcotesta, a similar compound to aryl, usually rich in lipids (Van Der Pijl, 1972). The presence of resources such as pulp is important for ant attraction (Rico-Gray & Oliveira, 2007).

Network analysis

The networks showed low connectance, a nested pattern, absence of significant modules and low specialization. These results indicate low interaction between diaspores and ants and with some species of plants and ants dominating most interactions. Other studies have shown that mutualistic networks between ants and diaspores were also nested (Guimarães et al., 2007; Anjos et al., 2018). In our study, we observed that some species of ants (e.g., *Pheidole* species) and plants (e.g., *G. guidonia*, *M. prasina*) concentrated most

of the interactions. This result agrees with those by Palacio et al. (2016), which showed that generalist species play a central role in highly diverse plant-frugivorous networks. In addition, the specialization values of the networks were very low (although the total and dry season networks were more specialized than the null models). This indicates that ants that explore diaspores are generalists and interacting with any diaspore type, just as plant species (diaspores) interact with several ant species. This is probably the result of opportunistic interactions (Anjos et al., 2018), and it is common in relationships with frugivorous insects (Passos & Oliveira, 2003), where most frugivorous species have generalist and opportunistic behavior and whose spectrum of fruits visited by different ant species commonly overlaps (Blüthgen, 2011). In the case of frugivorous vertebrates, specialization varies among dispersal groups and it is influenced by fruit characteristics (Donatti et al., 2011; García et al., 2018). The absence of differences in the values of nestedness, modularity and specialization between the rainy and dry networks indicates that ant-diaspore interactions remain stable despite differences in climate in our study area. Thus, species of ants and plants interact throughout the year, regardless of seasonal variation. In line with our results, Ruzi et al. (2017) found that the removal rate by ants for 12 species of neotropical pioneer trees was not affected by seasonality.

The core of generalist species was stable throughout the years. The *Pheidole transversostriata*, *Pheidole* sp3, *P. diligens*, *P. sigillata*, *P. pedana* and *Wasmannia auropunctata* species are almost always present in the generalist core, which makes them important species in the general structure of the network and within the community because they promote diaspore cleaning and removal. Core species are known to be competitively superior and to monopolize resources in interactions between ants and EFN-bearing plants (Dáttilo et al., 2013a; Dáttilo et al., 2014a, 2014c). A stable core appears to be robust to annual fluctuations, and core species tend to belong to lineages that are less volatile and/or generate multiple species in a short time span (Burin et al., 2021). Regarding the ant species in the core species in this study, *Pheidole* species are extremely competitive, and they monopolize the resources that they explore, being considered dominant omnivores (Silvestre et al., 2003), and *W. auropunctata* shows massive recruitment, which facilitates the control of the diaspore stations (Delabie et al., 2003; Silvestre et al., 2003). Species of the *Pheidole* and *Wasmannia* genera can remove seeds, although that is not frequent (Christianini et al., 2010). Thus, a core of interactions composed by such species may indicate high diaspore cleaning rates, which corroborates our results, as well as potential for removing diaspores. The presence of *P. striata* in the core of central species in the rainy season network may indicate an increase in removal by that species during rainy period, since it removes seeds (Christianini et al., 2010; Christianini et al., 2012). This idea should be evaluated in future studies.

Diaspore removal rate, destination and distance of diaspore removal

We observed high removal rates for two *Miconia* species and *I. edulis*, but not for *P. leiocarpa*. Differences in secondary removal rates occur among plant species (Christianini et al., 2012; Ruzi et al., 2017; Ortiz et al., 2021). The presence of several attractive resources for ants is one of the causes for such differences (Christianini et al., 2012; Ruzi et al., 2017; Clemente & Whitehead, 2019; Ortiz et al., 2021). The presence of secondary compounds, as occurs in *Psychotria* fruits, can reduce the ant recruitment and result in low removal rates (Cazetta et al., 2008; Santana et al., 2013). As a result, diaspores ‘preferred’ by ants have greater removal (e.g., *M. calvescens* = 65.8% of removal), and ‘non-preferred’ diaspores are rarely or not removed (e.g., *P. leiocarpa* = 2.1% of removal). This had already been observed in granivory by ants (Willet et al., 2000).

The studied ants removed diaspores to short distances. Removal distances are in accordance with data reported by Anjos et al. (2020), in which rainforests ecosystems show shorter removal distances by ants than savanna ecosystems. Hence, even at short distances diaspore removal decreases diaspore aggregation and helps the local plant population (Gorb & Gorb, 2000), as it may promote recruitment through a reduction in competition among seeds and a decrease in attacks by predators (Guimarães-Jr & Cogni, 2002). Despite their larger sizes (Table 1), *I. edulis* diaspores were removed for longer distances than those of *Miconia*. Diaspore size is also a key trait influencing removal rates (Pizo & Oliveira, 2001). Therefore, the high rates of removal of a large diaspore must have occurred due to the chemical composition of the fleshy portion. It is known that ants look for more fleshy fruits (Passos & Oliveira, 2003; Rico-Gray & Oliveira, 2007), and the composition of these fruits can also be an important factor (Pizo & Oliveira, 2001; Christianini et al., 2012).

Most of the removals of *I. edulis* and *M. calvescens* were to the nest, which is advantageous for seedling development (Farji-Brener & Medina, 2000). However, the arrival of many seeds in the nest can increase competition (Spiegel & Nathan, 2012). Therefore, behaviors in which ant species take some diaspores to the nest and abandons others can be advantageous for plants (Ortiz et al., 2021). In removals by *Atta sexdens*, nest deposition without subsequent seed discard may indicate that the diaspores serve as a substrate for the fungus and, therefore, dispersal does not occur. In the case of *M. calvescens*, in which the dispersal unit is the fruit with multiple seeds, some of them are abandoned along the way and can benefit from the removal distance. However, in *I. edulis*, in which the dispersal unit is the seed, *A. sexdens* may be acting as a predator.

General conclusions

In the Atlantic Forest, interactions between ants and diaspores are frequent and generalised, with ants playing an

important role in dispersal stages. In general, ants interact with diaspores by cleaning them, and some species also remove them. Ant-diaspore networks are generalist, nested and remain stable throughout the seasons. Thus, we can conclude that diaspore cleaning and removal occur continuously. The removal distance and final destination of diaspores depend on the diaspore species and on the ant species that remove them. Ants are good secondary dispersers for only some plant species, depending on their behavior and the identity of the ant species (Christianini et al., 2012; Clemente & Whitehead, 2019). For most plants, ants would play a more important role in cleaning and promoting germination (Pizo & Oliveira, 1998).

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