Received: 24 March 2020 | Accepted: 16 October 2020

DOI: 10.1111/1365-2745.13534

RESEARCH ARTICLE

Journal of Ecology BRITISH ECOLOGICAL SOCIETY

Extant fruit-eating birds promote genetically diverse seed rain, but disperse to fewer sites in defaunated tropical forests

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Funding information
CNPq, Grant/Award Number: 445353/2014-7; Fundação de Amparo à Pesquisa do Estado de São Paulo, Grant/Award Number: 2014/01029-5, 2016/22843-8, 2019/26436-6 and 2019/03005-0; Fundação para a Ciência e a Tecnologia, Grant/Award Number: IF/01375/2012

Handling Editor: Crystal McMichael

Abstract

1. The world-wide decline in populations of large-bodied vertebrates due to deforestation and poaching threatens the persistence of animal-dispersed plants by reducing long-distance seed dispersal and generating aggregated seed rain patterns.
2. We evaluated whether the composition of maternal trees contributing to the seed rain is also impacted by the loss of large frugivores. By combining molecular tools with a thorough sampling of the frugivore-generated seed rain we quantified the number of seeds, richness of maternal progenies and number of maternal effective alleles in the seed rain of a tropical palm *Euterpe edulis* across ten Atlantic Forest remnants with varying levels of avian defaunation and density of palm conspecifics.
3. Forest structure in defaunated areas was characterized by higher canopy openness. Defaunation did not affect the number of seeds dispersed or of effective alleles, but, together with palm density, was associated with higher numbers of maternal genotypes in the seed rain. This result suggests that medium-sized birds may play an important role in mixing maternal genotypes where large-sized frugivores have been extirpated. Defaunation, however, impacted the spatial distribution of seeds, with deposition sites in avian depauperated forests less likely to receive at least one seed.
4. *Synthesis*. Our study suggests that medium-sized frugivores contribute to maintaining the quantitative component of seed dispersal and local genetic diversity of a threatened tropical palm in human degraded forests and, therefore, may be important for guaranteeing the persistence of remnant animal-dispersed plant populations under scenarios of rapid environmental change. The loss of large-bodied frugivores, however, can disrupt longer dispersal events and strengthen the dispersal spatial limitation, with consequences for plant spatial distribution and fine-scale genetic structure at the population level.

KEYWORDS
allele diversity, anthropogenic defaunation, Atlantic forest, *Euterpe edulis*, frugivory, maternal progeny, microhabitat, thrushes

1 | INTRODUCTION

Populations of numerous large-bodied vertebrates have been extirpated or are in continuous decline world-wide as a result of extensive deforestation and poaching (Ceballos et al., 2017; Dirzo et al., 2014). Consequently, the persistence of animal-dispersed plants that inhabit animal-impoverished forests is threatened because they require the dispersal services provided by frugivorous vertebrates to complete their life cycle (Caughlin et al., 2014; Jordano, 2013; Kurten, 2013). Specifically, the loss of large frugivores may impair seed dispersal services if extant medium- to small-bodied vertebrates only provide suboptimal dispersal services because: (a) they often mobilize a reduced fraction of propagules (Holbrook & Loiselle, 2009); (b) they are not able to ingest large-sized seeds, and therefore, only medium- to small-sized seeds are dispersed (Carvalho, Lucas, & Côrtes, 2020; Galetti et al., 2013) and (c) they seldom reach distant sites, which reduces connectivity at the landscape level (Pérez-Méndez et al., 2016). As a result, the loss of frugivores may lead to seed dissemination limitation if extant dispersers are not sufficient to disperse all seeds away from the parent trees and beyond the genetic neighbourhood (i.e. demo-genetic limitation) and if seeds are dispersed in an aggregated fashion (i.e. spatially contagious limitation; Jordano, 2017; Jordano & Godoy, 2002; Schupp et al., 2002). For example, recent studies have shown that defaunation reduced the frequency of long-distance dispersal events (Caughlin et al., 2014; Pérez-Méndez et al., 2015). Yet, there is a lack of knowledge about the extent to which the richness of maternal progenies in the seed rain, that is the number of mother trees contributing to seed clumps in deposition sites, is impacted by the loss of large frugivores. This is important because the spatial distribution of the maternal progenies sets the initial genetic template from which dispersed seeds germinate and, eventually, get established (Browne & Karubian, 2016; García & Grivet, 2011). The mixture of unrelated maternal progenies can increase the probability of seedling survival, in turn contributing to the maintenance of high population genetic diversity (Browne & Karubian, 2016; Scofield et al., 2012). Therefore, evaluating how defaunation modifies the distribution of the maternal progenies in a frugivore-generated seed rain is crucial to forecast the probabilities of animal-dependent forest species to persist in an increasingly defaunated world.

Frugivores shape the seed rain in distinctive fashions according to their morphology and foraging behaviour (Choo et al., 2012; Côrtes & Uriarte, 2013; Karubian et al., 2010). As a result, both the quantitative (i.e. the number of removed seeds) and the qualitative components of seed dispersal (i.e. the dispersal distance and quality of deposition microsite) can be impacted by the composition of the frugivorous assemblages (Jordano & Schupp, 2000). For example, large frugivores move and disperse seeds to distant locations (Bueno et al., 2013; Holbrook & Loiselle, 2009), potentially bringing new maternal progenies from other genetic neighbourhoods or populations (García et al., 2007; Jordano, 2017; Jordano et al., 2007). Therefore, genetic diversity is expected to increase while genetic divergence is expected to decrease both at the seed rain and the population level (Karubian et al., 2015; Scofield et al., 2012). In contrast, small- and medium-bodied birds, such as thrushes, typically move locally and ingest few small seeds per visit (Galetti et al., 2013; Jordano et al., 2007), resulting in clumps of full- or half-sibling seeds (maternal progenies) dispersed nearby the mother tree (Caughlin et al., 2014; García et al., 2009). This foraging pattern reduces the local genetic diversity in the seed rain (Grivet et al., 2005) and may decrease population genetic diversity in the long term (Giombini et al., 2017). Additionally, frugivore behaviour is mediated by fine-scale environmental heterogeneity, which influences foraging and seed deposition patterns and imprints the spatial distribution of genetic variation in plant populations (García et al., 2009). For example, sites with high density of fruiting trees typically attract more frugivores, increasing seed removal and deposition (Alcántara et al., 2000; Carlo & Morales, 2008). At the same time, clumped distribution of fruiting trees might decrease seed dispersal distances because frugivores move short distances to forage on different maternal trees (Carlo & Morales, 2008). This contributes to the admixture of maternal progenies at the local scale that might maintain high genetic diversity among deposition sites within a population (García et al., 2009; Giombini et al., 2017). Therefore, the structure of forest canopy and particularly the density of fruiting conspecifics might enhance or dilute the impact of frugivore loss on the distribution of maternal progenies in the seed rain (Côrtes & Uriarte, 2013).

The extirpation of large-bodied avian frugivores has been previously associated with microevolutionary shifts in seed size (Carvalho, Lucas, & Côrtes, 2020; Galetti et al., 2013) and allele frequencies (Carvalho et al., 2016) in our study species, the neotropical palm *Euterpe edulis*. Here we characterize the spatial distribution of maternal progenies in a frugivore-generated seed rain of the palm *E. edulis* across areas subjected to different levels of frugivore loss. By combining molecular tools with a thorough sampling of the frugivore-generated seed rain we quantified the number of seeds, the richness of maternal palms contributing to the seed rain (i.e. maternal progenies) and the number of maternal effective alleles in pre-established deposition sites (Figure 1). We analysed these variables as a function of defaunation, density of conspecific fruiting palms and forest canopy structure. Small to medium-sized birds, mostly thrushes (*Turdus* spp.), visit palms more often than large birds and tend to remove fruits from multiple fruiting trees. We expect that the richness of maternal progenies will be negatively associated with defaunation because the lack of large frugivores hinders the input of new genotypes from beyond the immediate neighbourhood (Table 1). Because large frugivores are usually more mobile than medium-sized frugivores and disperse seeds to distant locations (Bueno et al., 2013; Holbrook & Loiselle, 2009), we also expect seed dispersal to be more spatially limited in defaunated areas (Pérez-Méndez et al., 2016), so that deposition sites are less likely to receive dispersed seeds. Also, we hypothesize that the richness of maternal progenies will reflect the local density of conspecific palms owing to the intense and local foraging activity of *Turdus* spp. (Table 1). Finally, we hypothesize that the amount of overlap in the composition of maternal progenies between deposition sites will be higher in forests with richer avian assemblages due to frugivores that act as mobile links among patches in the forest (Table 1). This is because large avian birds tend to ingest many fruits at once and can move long distances, promoting dispersal of siblings across multiple sites.

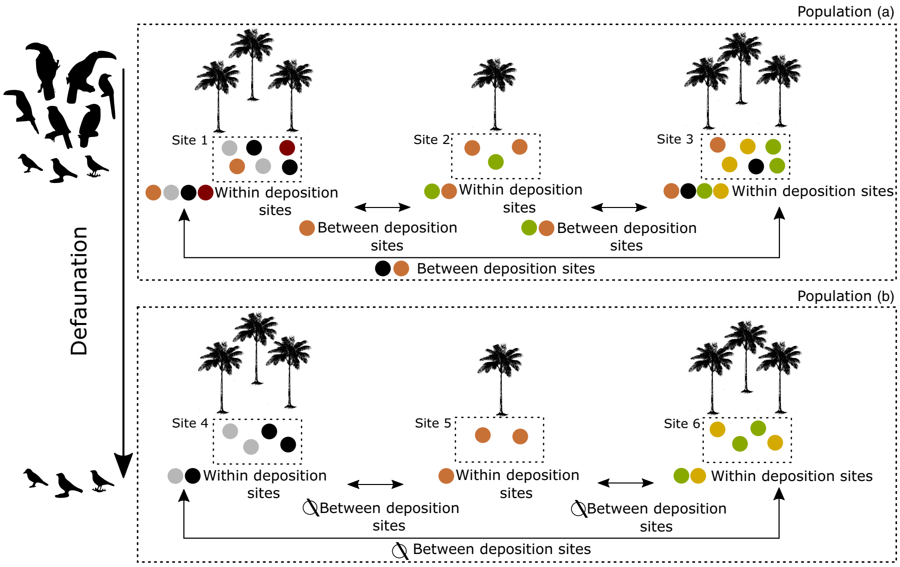
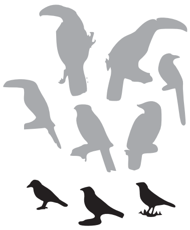


FIGURE 1 Scheme illustrating the expected richness of maternal progenies in frugivore-generated seed rains in forests with richer frugivore assemblages (a) and defaunated forest areas (b). Within-deposition sites represent the richness of maternal progenies; and between deposition sites represent the overlap of maternal progenies between pair of deposition sites. Circles represent dispersed seeds and colours indicate mother trees (i.e. seed sources). Therefore, circles of the same colour are progeny seeds from the same mother tree (i.e. siblings). Diagonally crossed circles indicate seed deposition sites that do not share maternal progenies

TABLE 1 Hypotheses of how defaunation, palm density and forest structure may influence the richness of maternal progenies, the number of effective alleles and the number of dispersed seeds of *Euterpe edulis*. (+) (–) and (=) mean positive, negative and no effect respectively. ✓ represents hypotheses that were corroborated

	Ecological theory	Expected effects on seed dispersal, maternal composition and genetic diversity in the seed rain
Greater defaunation 	Small birds are the most abundant in defaunated and non-defaunated areas. They ingest one or few seeds per visit but are frequent visitors. Defaunation may affect plant populations by decreasing seed dispersal distance but not the number of seeds being dispersed	(=) Number of dispersed seeds (–) Proportion of deposition sites ✓ that receive seeds (–) Richness of maternal progenies (–) Overlap of maternal progenies between seed deposition sites (–) Number of effective alleles
Greater palm density 	High density of fruiting plants may attract more birds increasing seed removal and seed deposition from different nearby tree sources	(+) Number of dispersed seeds ✓ (+) Richness of maternal progenies ✓ (+) Number of effective alleles
Denser forest structure 	Forest structure can determine seed rain patterns because of avoidance of frugivores to open habitats. Dense forest canopy may receive more dispersed seeds from different sources	(+) Number of dispersed seeds (+) Richness of maternal progenies (+) Number of effective alleles

2 | MATERIALS AND METHODS

2.1 | Study area and species

The Brazilian Atlantic Forest is an ideal system to test the effects of anthropogenic defaunation on ecological and evolutionary processes because it has been intensively disturbed by human activities for centuries; however, we can still find preserved areas along its distribution. The Atlantic Forest originally covered the Atlantic coast of Brazil extending from the Northeast to the Southern region of the continent, including parts of Argentina and Paraguay (Joly et al., 2014). Today it has been reduced to 12% of its original extension, which is estimated to be 150 million ha (Ribeiro et al., 2009). Habitat fragmentation, among other drivers, has caused a continuous and alarming loss of biodiversity during the last ~500 years, resulting in a landscape composed of natural habitat islands surrounded by croplands, pastures and urban matrix (Joly et al., 2014). The juçara palm *E. edulis* (Mart. Arecaceae) was once one of the dominant trees in the Atlantic forest but it is currently endangered and locally extinct in many areas owing to illegal harvesting of its edible meristem (Galetti & Fernandez, 1998). *Euterpe edulis* is a self-compatible monoecious species with predominantly outcrossed reproduction (Gaiotto et al., 2003) pollinated by small-sized bees (e.g. *Trigona spinipes*). This species bears fruits once a year with the fruiting season usually spanning around 3 months (Castro et al., 2007; Galetti et al., 1999). This palm produces ellipsoid fleshy fruits ranging from 6.7 to 16.6 mm in diameter (Galetti et al., 2013; Pizo et al., 2006), and is eaten by more than 58 birds and 20 mammalian species (Galetti et al., 2013). Yet, seeds are mostly dispersed by a reduced subset of large frugivorous birds such as cotingas *Procnias nudicollis*, toucans *Ramphastos* spp., and guans *Penelope* spp. and *Aburria jacutinga* and medium-sized avian frugivores *Turdus* spp. (Bello et al., 2017; Galetti et al., 2013). Historically, our study areas shared a similar assemblage of seed dispersers, but forest fragmentation and hunting have impoverished the assemblage of large vertebrates leading to frugivore downsizing across the Atlantic Forest (Galetti et al., 2013). There are no data available on when defaunation happened in our study areas, but it is known that deforestation in this region dates back to the 1800s, through the conversion of continuous forest into agricultural field (mainly coffee; Dean, 1976 apud Galetti et al., 2013).

2.2 | Sampling design

We selected ten study areas in the southeastern region of the Brazilian Atlantic Forest (Figure 2; Table S1). In each area we set and georeferenced (with a Garmin GPS) 15 deposition sites (Figure S1) that consisted of two seed traps of 1 × 1 m placed side by side and suspended 1 m above the ground. The deposition sites were distributed along transects in each study area. We assured that each deposition site was placed at least 50 m apart from each other and not directly below a fruiting palm to prevent collection of fruits not dispersed by frugivores. Density of fruiting palms in the immediate vicinities (within 8-m radius) of the deposition sites varied widely in all study areas (from 0 to 26 fruiting palms per vicinities). Seed traps were monitored and dispersed seeds collected every 40 days during the fruiting season of *E. edulis*, which usually spans around 3 months from April to September depending on the area (Galetti et al., 1999). Seeds were sampled from four consecutive fruiting seasons (years 2013–2016). Dispersed seeds were collected and stored in a paper bag until further analysis in the laboratory.

To characterize the local environmental heterogeneity of the forest canopy we set an 8-m radius centred in between each pair of seed traps (i.e. deposition site) to measure two sets of variables. This radius was chosen because a study using eleven 25 × 50 m plots from six areas along the Atlantic Forest showed that most *E. edulis* seedlings are within 8 m of the nearest adult palm (adult stems), which may be evidence that the bulk of seed dispersal events happens within this interval (Valverde et al., 2020). The first set of variables characterizes the conspecific neighbourhood, including: (a) the number of fruiting palms; and (b) the distance to the nearest fruiting palm. These variables were evaluated once a year and, because they did not vary considerably across years, we used the minimum distance to the nearest fruiting palm and the maximum number of fruiting palm over the years. It was possible to identify all individuals that fruited in each season because the empty infructescence remains attached to the palm for a long period. The second set of variables depicts forest canopy structure in terms of: (a) distance to the nearest gap opening; (b) number of total trees with dbh > 10 cm; (c) and dbh > 30 cm; (d) percentage of open canopy above deposition sites;

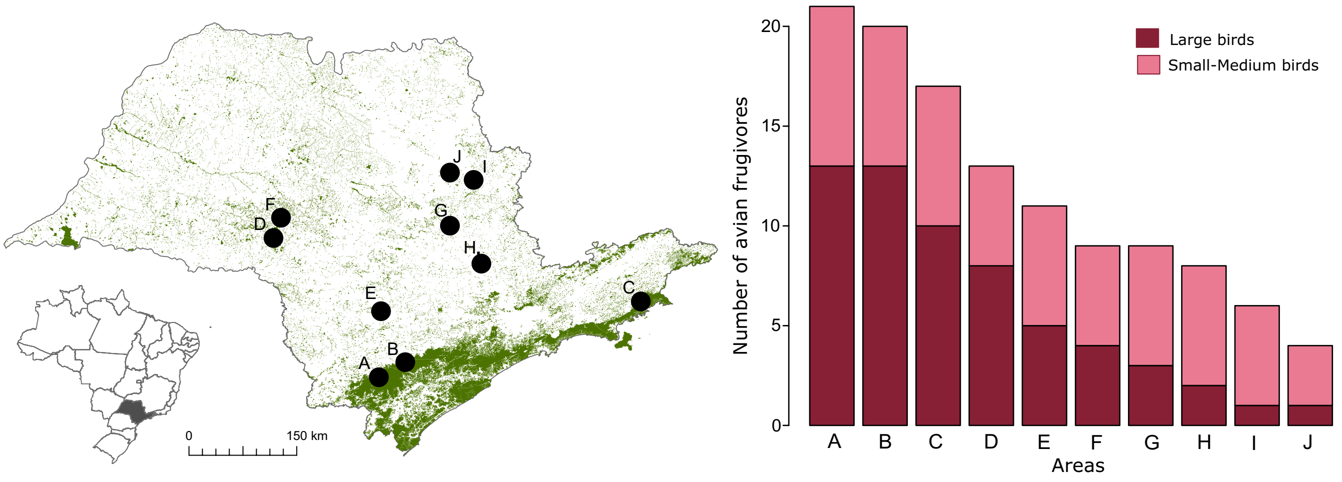


FIGURE 2 Geographic distribution of ten study areas where we characterized seed deposition patterns and the richness of the maternal progenies of *Euterpe edulis*in the Brazilian Atlantic Forest. The São Paulo state is outlined in the inset with Brazil's map and states; remnants of Atlantic Forest are represented in green. Codes indicate the level of defaunation with area A harbouring the most complete frugivore assemblage and J the most defaunated assemblage lacking large fruit-eating birds. Bar plot represents the number of avian frugivores that disperse seeds of *E. edulis*in each study area (dark pink representing large birds and light pink small-medium birds). Large birds are the avian frugivores with gape sizes wider than 16.6 mm, which is the maximum fruit diameter found for *E. edulis*

(e) leaf area index (LAI); and (f) canopy height. These variables were measured only once in the first year of sampling. We considered as 'gap opening' an area with partial or complete removal of tree crowns, leading to a higher incidence of light. Percentage of open canopy and LAI were estimated from hemispherical photography with Gap Light Analyzer—GLA (Frazer et al., 1999). The hemispherical photographs were taken with a Nikon Fisheye Converter FC-E8 (Nikon) and the camera was placed at the centre of each deposition site, 1.30 m above the ground and oriented so that the top of each photograph pointed north. The number of fruiting palms and distance to the nearest fruiting palm were highly correlated ($r = -0.62$, $p < 0.001$), thus we retained only the first variable in further analysis. To depict forest structure, we applied a principal component analysis (PCA) to a matrix containing all above-mentioned variables (columns) per deposition sites (rows). We retained the first PCA axis (PCA1) that explained 40% of the total variance. Positive values of PCA1 represent deposition sites with denser and higher forest canopy, while negative values indicate sites with open canopy (Figure S2). PCA analysis was performed with the stats package (v 3.4.0) implemented in R (v 3.4.0; R Core Team, 2014).

2.3 | Defaunation level

We obtained the composition of the avian disperser community in each area based on frugivory observations and census studies (Bello et al., 2017; Galetti et al., 2013). The defaunation level (D) was measured as the difference between the number of species of seed dispersers in the richest avian assemblage (Site A, $n = 21$, $D = 0$) and the number of species of seed dispersers found at the focal area, with area J being the most defaunated ($n = 4$, $D = 17$; Table 2; Figure 2). Thus, defaunation levels were based on presence–absence data only. Despite the loss of some large-sized frugivore birds, areas A–C presented very similar defaunation levels ranging between 0 and 4. The frugivore assemblages of these study areas were composed on average by 20 frugivores that included families of large birds such as Ramphastidae, Trogonidae, Contigidae and Momotidae; and medium-sized birds of the Turdidae family (Figure 2; Table S3). Study areas D-F presented intermediate defaunation values. The most defaunated areas were study areas G–J, with frugivore assemblages mainly including species of the Turdidae family (i.e. thrushes). However, this index is still useful as it reflects the loss of large birds across the areas, enabling us to test our hypotheses. Estimating species abundance for the entire frugivore assemblage in tropical areas is not an easy task, because many species are rare, cryptic, and the vegetation structure makes it difficult to locate.

2.4 | DNA extraction and genotyping

We identified the genotype of the mother tree for each dispersed seed (maternal progeny) by genotyping its endocarp (i.e. maternal tissue; Godoy & Jordano, 2001). We attempted to genotype at least ten seeds from each deposition site, but we failed to obtain this number in some deposition sites either because there were fewer seeds available or due to poor DNA amplification. Overall, we extracted DNA from 1,330 endocarps following the protocol described by Lucas et al. (2019). All endocarps were genotyped using seven highly polymorphic microsatellite loci (EE3, EE23, EE25, EE45, EE47, EE52 and EE54; Gaiotto et al., 2001), following the PCR protocol described by Lucas et al. (2019). DNA fragments were sized in ABI Prism 3100 automated DNA sequencer (Applied Biosystems) using GeneScan Rox 500 size standard (Applied Biosystems), and scored using GeneMapper v.4.1 software (Applied Biosystems). We

TABLE 2 Characterization of the seed deposition and maternal progenies in the seed rain of *Euterpe edulis* in each study area. For each study area we report the defaunation level; fruiting palm density; mean number of sampled dispersed seeds per deposition site; percentage of the 15 deposition sites that did not receive any dispersed seeds; total number of genotyped endocarps; total number of maternal progenies per study area; mean number of different maternal progenies per deposition sites in the study area. The names of study areas are in Table S1

Study area	Defaunation level	Fruiting palm density	Mean *N* of dispersed seeds (*SD*)	% of empty deposition sites	*N* of genotyped endocarps	Total *N* of different maternal progenies	Mean *N* of different maternal progenies (*SD*)
A	0	8.33	13.00 (9.55)	0	98	52	3.46 (1.30)
B	1	0.80	2.75 (4.22)	33	57	27	1.80 (1.69)
C	4	8.13	5.06 (3.33)	0	119	71	4.86 (2.47)
D	8	1.20	2.07 (4.23)	46	32	20	1.33 (1.44)
E	10	2.15	7.19 (9.69)	8	23	16	1.61 (1.44)
F	12	3.80	4.89 (5.73)	33	52	30	2.20 (1.74)
G	12	3.60	31.26 (57.83)	20	36	24	1.73 (1.57)
H	13	10.8	25.4 (31.36)	0	148	93	6.20 (2.39)
I	15	1.93	4.31 (4.13)	7	77	53	3.53 (1.92)
J	17	0.86	1.15 (2.39)	80	4	4	0.40 (0.73)

repeated all dubious genotypes and included only those that were scored without doubt in the analysis. In addition, we included a positive control that was unambiguously assigned to the same genotype in all fragment size analyses.

We recorded a high rate of amplification failure possibly due to DNA degradation, contamination from fungal or microbial DNA or the presence of polymerase inhibitors, such as humic acids. These recurring failures led to a high number of incomplete multilocus genotypes. To avoid excluding incomplete multilocus genotypes we defined the minimum number of loci necessary to distinguish individuals with high confidence (Supporting Information 4: Figure S4; Table S4). We used complete multilocus genotypes of 296 adult palms from five study areas (two defaunated and three with richer frugivore assemblages) to infer the probability of identity as a function of the number of loci and determine the minimum number of loci for which no two individuals share the same genotypes (Waits et al., 2001). We found that a minimum of six completely scored loci were sufficient to obtain distinct multilocus genotypes for the set of 296 adults. Six hundred and forty-six endocarps out of 1,330 (48%) were successfully genotyped for at least six loci, and all subsequent analyses were performed using the set of 646 endocarps (mean of 5.71 [3.75 *SD*] seeds analysed per deposition site). For these endocarps, all microsatellite loci showed polymorphism ranging from 19 to 27 alleles (Table S4). We used Microchecker v 2.2.3 (Van Oosterhout et al., 2004) to search for genotyping errors and we used ML-Null Freq (http://www.montana.edu/kalinowski/software/null-freq.html) to infer the frequency of null alleles using maximum-likelihood estimation (Kalinowski & Taper, 2006) that varied between 0.02 and 0.07 across loci (Table S4). The overall missing-data load of our final dataset was 2.2%.

2.5 | Dispersal and allelic diversity analysis

We identified maternal progenies (i.e. seeds from the same mother tree) by matching the multilocus genotypes of dispersed endocarps using the R package ALLELEMATCH v 2.5 (Galpern et al., 2012), allowing one allele mismatching to account for the possibility of genotyping errors that are expected when genotyping low-quality DNA sources, such as dispersed endocarp tissues (García et al., 2009; Lucas et al., 2019). Therefore, multilocus genotypes that shared at least 13 alleles out of 14 were considered identical and were assigned to a single maternal source. We have not repeated a subset of individuals to estimate the rate of genotyping error. However, we estimate that the mismatch at one allele corresponds to a genotyping error of 11% estimated using a similar set of loci (Santos et al., 2018). To characterize the richness of maternal progenies within and across deposition sites, we estimated the number of different maternal genotypes at the deposition site and the mean at the study area levels respectively. Moreover, we quantified the amount of overlap in the maternal progenies between deposition sites (the number of mothers contributing simultaneously to pairs of deposition sites). Finally, we used the software GenAlEx 6.5 (Peakall & Smouse, 2012) to estimate the effective number of maternal alleles within deposition sites and the mean across sites at the study area level.

2.6 | Influence of fruiting palm density, forest structure and defaunation on seed deposition, maternal progenies richness and genetic diversity patterns

The influence of fruiting palm density, forest structure and defaunation on seed deposition patterns and seed rain genetic diversity were evaluated at the level of deposition site ($n = 150$) and study area ($n = 10$). We performed these analyses at two levels because the variables were represented in different scales: whereas defaunation values are unique measurements per study area, density of fruiting palms and forest structure characterize the microhabitat around deposition sites. Three models were tested at the microhabitat level, as follows: *seed dispersal model*- the effect of fruiting palm density and forest structure (PCA1) on the number of dispersed seeds; *maternal progeny model*- the effect of fruiting palm density, forest structure (PCA1) and number of dispersed seeds on the number of maternal genotypes (maternal progenies); and *allele dispersal model*- the effect of fruiting palm density, forest structure and number of dispersed seeds on the number of maternal effective alleles. We fitted full models using zero inflated models because of the high proportion of zero values in all response variables. We used the maximum-likelihood method using R package GLMMTMB (Magnusson et al., 2017), with the study area treated as random effect and with a single zero-inflation parameter applied to all observations (ziformula = ~1). Seed, maternal progeny and allele dispersal models were fitted using negative binomial, Poisson and normal error distributions respectively. The negative binomial and Poisson error distributions were used because number of seeds and number of maternal genotypes are discrete counting data. Because seed dispersal models showed overdispersion, we used negative binomial error distribution. The normal error distribution was used in allele dispersal models because the number of maternal effective alleles is a non-discrete variable. At the study area level, we tested five models. Instead of using data of individual deposition sites, we used the mean number of dispersed seeds, mean number of maternal genotypes and mean number of effective maternal alleles across deposition sites, the proportion of deposition sites that received at least one seed per area, the density of fruiting palms per area and the defaunation level per area. The models were constructed as follows: two *seed dispersal models*—the effect of density of fruiting palms and defaunation level on the mean number of dispersed seeds per area and on the proportion of deposition sites that received at least one seed per area; one *maternal progeny model*—the effect of fruiting palm density, number of dispersed seeds and defaunation level on the mean number of maternal genotypes per area; one *allele dispersal model*—the effect of fruiting palm density, number of dispersed seeds and defaunation level on the mean number of

TABLE 3 Summary of the microhabitat and study area models showing the best-fitting models (ΔAIC ≤ 2) for seed deposition, richness of maternal progenies and effective maternal alleles in the seed rain of *Euterpe edulis* in 150 deposition sites across ten study areas in the Brazilian Atlantic Forest. Response variables are: number of dispersed seeds, number of different maternal genotypes and number of effective alleles per deposition site (microhabitat-level models); mean number of dispersed seeds, different maternal genotypes and effective alleles across deposition sites; the proportion of deposition sites that received at least one seed; and defaunation level (study area-level models). The predictors differ among models and may include the number of dispersed seeds, density of fruiting palms, forest canopy structure and defaunation level. The null model represents the absence of an effect; *K*, the number of estimated parameters; AICc, Akaike corrected for small samples and wAICc, Akaike's weight of evidence that were calculated across all considered models. Likelihood Ratio Test (LRT) was performed to assess if each predictor variable significantly improved the model's log likelihood. Significance levels are highlighted with *$p < 0.05$. Information about these models is available in Table S5a in the Supporting Information

	Response	Model	*K*	AICc	ΔAICc	wAICc
Microhabitat models	*N* of dispersed seeds	Fruiting palm density*	5	879.1	0.00	0.52
		Fruiting palm density* + Forest canopy structure	6	879.3	0.19	0.48
	N of different maternal genotypes	Null model	3	443.1	0.00	0.37
		N of dispersed seeds	4	444.9	1.75	0.16
		Forest canopy structure	4	445.0	1.87	0.15
	N of effective alleles	*N* dispersed seeds*	5	369.8	0.00	0.45
		Fruiting palm density + *N* dispersed seeds*	6	371.5	1.71	0.19
Study area models	Mean *N* of dispersed seeds	Null model	2	80.0	0.00	0.56
		Fruiting palm density	3	80.9	0.97	0.34
	Proportion of deposition sites that received at least one seed	Fruiting palm density* + Defaunation*	3	57.3	0.00	0.69
		Fruiting palm density*	2	58.9	1.59	0.31
	Mean *N* of different maternal genotypes	Fruiting palm density*	3	29.2	0.00	0.51
		Fruiting palm density* + Defaunation*	4	29.6	0.41	0.41
	Mean *N* of effective alleles	Fruiting palm density* + Defaunation*	4	19.0	0.00	0.39
		Null model	2	19.7	0.69	0.27
		Fruiting palm density	3	20.9	1.89	0.15
	Defaunation	Forest canopy structure*	3	60.9	0.00	0.92

effective alleles per area; and one *defaunation model*—the defaunation level was modelled as a function of forest structure (average PCA1 across deposition sites) and fruiting palm density. Forest structure was not included in the seed dispersal, maternal progeny and allele dispersal models at the study area level because it was associated with defaunation level, as indicated by the results of the defaunation model above. Except for the variable of proportion of deposition sites that received at least one seed per area that was modelled with binomial distribution, all study area models were fitted using linear models with normal error distributions and the maximum-likelihood method.

Because the number of genotyped seeds used to quantify the number of maternal progenies differed across deposition sites, we included the number of genotyped seeds as an *offset* parameter in all models. An offset variable is one that is treated like a regression covariate whose parameter is fixed to be 1.0. It is useful when the response variable is estimated from different sample sizes, as was the case for the variable richness of maternal genotypes. Starting with the full model, we fitted nested models with all possible combinations of the fixed effects using the *dredge* function from R package MuMIn (Barton & Barton, 2018), and then compared all models using AICc. The set of best models (ΔAICc ≤ 2) were compared to reduced models without each predictor variable using likelihood ratio tests (LRT, $\alpha = 0.05$). Significance levels of LRT ($p < 0.05$) are highlighted with * in the Table 3. Finally, the results of best models were plotted using the EFFECTS R package (Fox, 2003).

3 | RESULTS

3.1 | Influence of fruiting palm density, forest structure and defaunation on seed deposition patterns

The number of dispersed seeds varied across deposition sites from zero (23% of deposition sites) to a maximum of 325 (Table 2; Figure S5a,b). Overall, 53% of the deposition sites received between one and 10 seeds and 23% collected more than 11 seeds.

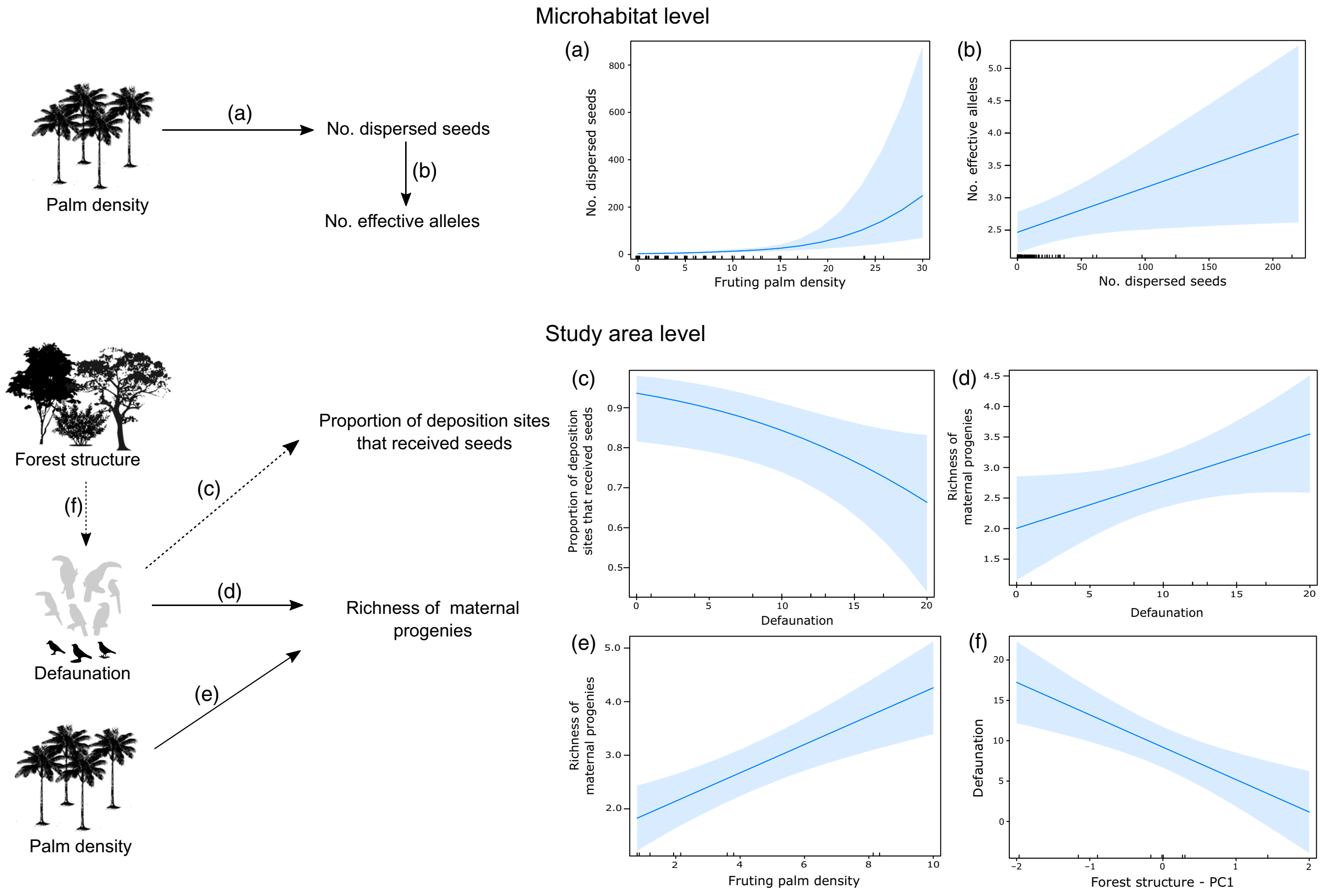


FIGURE 3 Results summary and effect plots (a–f) of the influence of density of fruiting palms, forest structure and defaunation on seed deposition, maternal progenies and effective alleles patterns of *Euterpe edulis*at the microhabitat and study area levels in the Atlantic Forest. Solid and dashed arrows indicate positive and negative statistically significant associations respectively. The blue band is a 95% confidence envelope for the estimated coefficients

The number of dispersed seeds per deposition site was positively affected by local palm density, but was not influenced by the level of defaunation nor forest structure (Table 3; Figure 3a; Table S5a,b). We found, however, that deposition sites in more defaunated areas were less likely to receive at least one seed (p-value for LRT = 0.01; Table 3; Figure 3c; Table S5a,b), indicating that there are more unvisited patches in areas with fewer disperser species, which suggests dispersal spatial limitation.

3.2 | Influence of fruiting palm density, forest structure and defaunation on maternal progenies richness and genetic diversity patterns

In total, we identified 390 mother trees (different maternal genotypes) contributing to the sampled seed rain of 646 seeds (Table 2). The number of mother trees contributing to the seed rain varied widely among deposition sites and across study areas (Table 2; Figure S5b). The number of different maternal genotypes across the deposition sites varied from 0 to 12 in all areas. We observed that most of the contributing mother trees contributed with a single seed dispersal event (68.2%), but some trees contributed with up to 12 seeds in areas with richer frugivore assemblage (Figure S5b). At the microhabitat level, the null model was among the plausible candidate models indicating that the number of different maternal genotypes was not influenced by any of the tested covariates (number of dispersed seeds, fruiting palm density and forest structure; Table 3; Table S5b). At the study area, however, the mean number of maternal genotypes was positively influenced by the level of defaunation (p-value for LRT = 0.01) and the fruiting palm density (p-value for LRT = 0.001 and 0.0001; Table 3; Figure 3d,e; Table S5a,b). The level of defaunation was present in only one model in combination with palm density. The number of effective alleles was not influenced by any of the covariates at the study area level; however, it was influenced by the number of dispersed seeds at the microhabitat level (p-value for LRT = 0.03; Table 3; Figure 3b; Table S5a,b). Finally, the defaunation level was negatively associated with the forest canopy structure, with denser and higher forest canopy associated with higher richness of frugivores (p-value for LRT = 0.0006; Table 3; Figure 3f; Table S5a,b).

In general, each deposition site contained seeds from a distinctive pool of mothers, suggesting that maternal progenies are highly spatially structured in all areas. We only found five instances of shared maternal progenies between deposition sites, all occurring in areas with large-bodied frugivores (Figure S1).

4 | DISCUSSION

In this study we aimed to disentangle the effect of defaunation, palm density and forest structure factors in determining the seed deposition patterns across human-dominated Atlantic forests. We used polymorphic microsatellite markers to assess the maternal composition and genetic diversity in the seed rain of *E. edulis* in ten forest remnants under distinct levels of avian frugivore defaunation. Our hypotheses were partially corroborated by our results (Table 1). In general, our results indicate that medium-sized birds may have an important role in providing dispersal services to *E. edulis*, because even in the absence of large seed dispersers, a sizeable quantity of seeds from multiple fruiting trees is dispersed. This is particularly important for the maintenance of high population genetic diversity. However, the fact that deposition sites in more defaunated areas were less likely to receive at least one seed is evidence of spatial seed limitation owing to the loss of large frugivores that are more likely to move seeds beyond the immediate maternal neighborhood (Jordano, 2017; Jordano & Godoy, 2002; Schupp et al., 2002).

4.1 | Influence of defaunation on seed deposition patterns and maternal progenies richness in the seed rain

Richer assemblages of avian frugivores, including species of medium- and large-sized frugivores, were more prone to occur in areas with denser and higher forest canopy. Similar results have been found in other Atlantic forest areas showing that the diversity of forest-dependent birds decreases with the simplification of vegetation complexity (Morante-Filho et al., 2018). It is possible that higher vegetation complexity provides higher availability of food resources (Morante-Filho et al., 2018) or that hunting pressure covaries with selective logging that simplifies the vegetation complexity.

Our results show that plant populations lacking large-sized dispersers still receive quantitatively efficient dispersal services (i.e. quantity of dispersed seeds sensus Schupp et al., 2002) when an active set of medium-sized frugivores is still in place. This pattern was found in other studies that report medium-sized seeded plants being dispersed by extant small- and medium-bodied frugivores in degraded habitats (Farwig et al., 2017; Pérez-Méndez et al., 2015). Previous studies have attributed this result to competition release caused by the extirpation of the large seed dispersers, which results in augmented densities of small- and medium-sized frugivores (Farwig et al., 2017; Kurten, 2013). In our study, this result can be attributed to the fact that medium-sized frugivores are, quantitively, the most effective *E. edulis* seed dispersers in defaunated and pristine forest (Carvalho et al., 2019; Galetti et al., 2013; Rother et al., 2016). Thus, the loss of large frugivores might not impact the amount of seed being dispersed. In contrast to quantitative aspects, our results showed that the loss of large frugivores reduced some qualitative aspects of dispersal services. Deposition sites in areas with impoverished fauna were less likely to receive at least one seed, indicating that dispersal is more spatially limited. The spatial dispersal limitation can preclude seeds to reach and colonize new microhabitats and, therefore, intensify plant aggregation over time (Schupp et al., 2002).

The distribution of maternal progenies in the seed rain can be explained by differences in foraging behaviour between medium- and large-sized frugivores. Thrushes *Turdus* spp. are the main seed dispersers of *E. edulis* (Galetti et al., 2013; Rother et al., 2016) because they occur in pristine and degraded habitats and are the species that remove the largest amount of seeds. Their small body and gape sizes allow thrushes to consume and disperse only one or two seeds per visit when they forage on *E. edulis* (Galetti et al., 2013; Rother et al., 2016). Their limited movement patterns and fast gut retention time (Morales et al., 2013) result in local dispersal patterns (García et al., 2009; Jordano, 2017). Therefore, thrushes most likely generate seed rains with high number of mother trees at the microhabitat and study area levels. In contrast, large-sized frugivores, only found in more preserved areas, consume large quantities of fruits per visit (Galetti et al., 2013; Rother et al., 2016) and, therefore, are more likely to disperse numerous sibs propagules to the same deposition site (Jordano, 2017). Consequently, the average number of maternal genotypes was not negatively impacted by defaunation. These results indicate that medium-sized, abundant frugivores, are key to maintaining local genetic diversity in areas subjected to different levels of human-driven perturbation. The mixture of maternal progenies provided by these frugivores may be crucial to the local demographic cycle of *E. edulis* because experimental studies have documented influences of the genetic relatedness among neighbour seeds on germination and seedling survival rates (Browne & Karubian, 2016; Scofield et al., 2012). Nevertheless, it is worth mentioning that, because of high seed size variation, small seeds of *E. edulis* can still be dispersed in defaunated areas (Carvalho, Lucas, & Côrtes, 2020). It is important to note, however, that different outcomes would be expected for larger-seeded plant species that can only be dispersed by larger-bodied frugivores. In the Atlantic Forest, for example, species with seed length >16 mm represent about 28% of vertebrate-dispersed species (Bello et al., 2017). For these species, medium-sized frugivores are not able to compensate for the loss of larger frugivores and we would expect a decrease or collapse of seed dispersal at local and landscape scale (Pérez-Méndez et al., 2016, 2018).

Overall, each deposition site contained seeds from a different set of mothers, suggesting that maternal progenies are highly spatially structured in all areas. We did find, however, a few events of shared maternal progeny between deposition sites in areas with large-bodied frugivores, which may indicate overlap in spatially ample seed shadows generated by these birds. These results suggest that large frugivores are able to move and disperse seeds of *E. edulis* beyond the immediate vicinities of maternal plant, promoting small scale gene flow between microsites. More defaunated areas did not present any sharing of siblings between deposition sites, suggesting that defaunation of large-bodied frugivores lead to the collapse of movements away from the genetic neighbourhood (Jordano, 2017). Thus, although medium-sized frugivores are able to maintain local genetic diversity in fragmented and animal-impoverished landscapes (Carvalho et al., 2016), the loss of large frugivores imposes a dispersal limitation in distance for this species, which may have lasting effects on gene flow and genetic structure (Carvalho et al., 2016; Santos et al., 2016). It is undeniable, however, that we could have observed more instances of shared progenies among deposition sites if sampling was to be continued.

Because medium-sized frugivores are by far the most effective seed disperser in defaunated forests (Carvalho et al., 2019; Galetti et al., 2013), we could indirectly attribute seed and genotype dispersal patterns to these species. However, the same is not possible in pristine forests because of the high diversity of frugivores contributing to dispersal and the lack of species-specific diagnosable signs left on dispersed seeds. Thus, to effectively understand the contribution of each seed disperser to the seed rain future studies may take advantage of molecular tools, such as DNA barcoding, to identify the frugivores of each dispersed seed (González-Varo et al., 2017).

4.2 | Fruiting palm density influences seed deposition patterns and maternal progenies richness in the seed rain

Our results also showed a positive effect of fruiting palm density on the number of dispersed seeds and maternal progenies at the microhabitat and/or study area levels. This effect can be a result of overlapping seed shadows of nearby palm conspecifics due to short dispersal distances (Alcántara et al., 2000; Schupp et al., 2002) or the attraction of seed dispersers towards sites with high availability of fruit resources (Carlo & Morales, 2008) leading to contagious dispersal limitation (Schupp et al., 2002). At this moment is difficult to tease both processes apart because we are still conducting maternity analysis to precisely assign seeds to the most probable mother tree within the deposition site vicinities. It is important, however, to emphasize that the density of adults of *E. edulis* has been decreasing in most of forests due to illegal harvesting of its edible meristem (Galetti & Fernandez, 1998). Therefore, we highlight the importance of maintaining high densities of *E. edulis* to prevent the loss of local genetic diversity (Carvalho et al., 2019).

5 | CONCLUSIONS

We suggest that medium-sized frugivores disperse large amount of seeds and contribute to the maintenance of high local genetic diversity in human degraded forests. Nevertheless, the loss of large-sized seed dispersers impacted the quality of seed dispersal service, mainly in terms of dispersal away from the local neighbourhood, with anticipated negative effects in the long term for the persistence of a key palm tropical tree *E. edulis*. Our results are consistent with previous findings on the effect of defaunation on genetic diversity and differentiation of *E. edulis* populations (Carvalho et al., 2015, 2016, 2019). Previously, we reported that the functional loss of large seed dispersers resulted in genetic differentiation among *E. edulis* populations; however, unexpectedly, we did not find evidence that defaunation would reduce genetic diversity or increased fine-spatial genetic structure at the seedling stages (Carvalho et al., 2015, 2016). Because we lacked understanding about the fine-scale processes generating spatial and genetic distribution of *E. edulis*, these patterns were attributed to time-lag effects between defaunation and genetic changes. The present results indicate that the maintenance of genetic diversity in frugivore impoverished areas may be driven by the dispersal activity of remnant medium-sized frugivores in dispersing seeds of different mother trees to the same deposition site. Thus, our study highlights that medium-sized frugivores contribute to ecological services to a threatened tropical palm and, therefore, may be critical for promoting evolutionary endurance under scenarios of rapid environmental change.

ACKNOWLEDGEMENTS

This work was supported by the competitive grant CNPq 445353/2014-7 that we gratefully acknowledge. C.d.S.C. received Fundação de Amparo à Pesquisa do Estado de São Paulo Scholarships (FAPESP, no. 2014/01029-5, 2016/22843-8 and 2019/26436-6). We thank S Nazareth and several interns for field and laboratory assistance, Fundação Florestal and IBAMA for permission to sample in State Reserves and Federal Reserves and many farm owners for permission to sample in private properties. We also thank M. Galetti for reviewing this manuscript. C.G. was supported by the IF Investigator Programme (IF/01375/2012) funded by the Fundação para a Ciência e a Tecnologia (FCT). M.C.C. was supported by grant FAPESP (2019/03005-0).

AUTHORS' CONTRIBUTIONS

C.d.S.C. and M.C.C. conceptualized and performed the study design; C.d.S.C. collected, analysed the data and wrote the manuscript; M.S.L. helped in collecting samples and contributed to writing the manuscript; C.G., M.C.C. and P.J. contributed to data analyses and in writing the manuscript.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/1365-2745.13534.

DATA AVAILABILITY STATEMENT

Data available via the Figshare Repository: https://doi.org/10.6084/m9.figshare.13129913 (Carvalho et al., 2020a). All the mentioned R scripts are available in the Zenodo Repository: https://doi.org/10.5281/zenodo.4118367 (Carvalho et al., 2020b).

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SUPPORTING INFORMATION

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

How to cite this article: Carvalho CS, García C, Lucas MS, Jordano P, Côrtes MC. Extant fruit-eating birds promote genetically diverse seed rain, but disperse to fewer sites in defaunated tropical forests. *J Ecol*. 2021;109:1055–1067. https://doi.org/10.1111/1365-2745.13534