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

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Abstract

The worldwide 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. 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. 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. *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.

Introduction

Populations of numerous large-bodied vertebrates have been extirpated or are in continuous decline worldwide as a result of extensive deforestation and poaching (Ceballos, Ehrlich, & Dirzo, 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: (i) they often mobilize a reduced fraction of propagules (Holbrook & Loiselle, 2009); (ii) they are not able to ingest large-sized seeds, and therefore, only medium- to small-sized seeds are dispersed (Galetti et al., 2013; Carvalho, Souza, & Côrtes accepted); and (iii) they seldom reach distant sites, which reduces connectivity at the landscape level (Pérez-Méndez, Jordano, García, & Valido, 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 neighborhood (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, Milleron, & Russo, 2002). For example, recent studies have shown that defaunation reduced the frequency of long-distance dispersal events (Caughlin et al., 2014; Pérez-Méndez, Jordano, & Valido, 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, Smouse, Karubian, & Sork, 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 behavior (Choo, Juenger, & Simpson, 2012; Côrtes & Uriarte, 2013; Karubian, Sork,

84 Roorda, Durães, & Smith, 2010). As a result, both the quantitative (i.e. the number of removed

85 seeds) and the qualitative components of seed dispersal (i.e. the dispersal distance and quality of

86 deposition microsite) can be impacted by the composition of the frugivorous assemblages (Jordano

87 & Schupp, 2000). For example, large frugivores move and disperse seeds to distant locations

88 (Bueno et al., 2013; Holbrook & Loiselle, 2009), potentially bringing new maternal progenies from

89 other genetic neighborhoods or populations (García, Jordano, & Godoy, 2007; Jordano, 2017;

90 Jordano, Garcia, Godoy, & Garcia-Castano, 2007). Therefore, genetic diversity is expected to

91 increase while genetic divergence is expected to decrease both at the seed rain and the population

92 level (Karubian, Ottewell, Link, & Di Fiore, 2015; Scofield et al., 2012). In contrast, small- and

93 medium-bodied birds, such as thrushes, typically move locally and ingest few small seeds per visit

94 (Galetti et al., 2013; Jordano et al., 2007), resulting in clumps of full- or half-sibling seeds (maternal

95 progenies) dispersed nearby the mother tree (Caughlin et al., 2014; García, Jordano, Arroyo, &

96 Godoy, 2009). This foraging pattern reduces the local genetic diversity in the seed rain (Grivet,

97 Smouse, & Sork, 2005) and may decrease population genetic diversity in the long term (Giombini,

98 Bravo, Sica, & Tosto, 2017). Additionally, frugivore behavior is mediated by fine-scale

99 environmental heterogeneity, which influences foraging and seed deposition patterns and imprints

100 the spatial distribution of genetic variation in plant populations (García et al., 2009). For example,

101 sites with high density of fruiting trees typically attract more frugivores, increasing seed removal

102 and deposition (Alcántara, Rey, Valera, & Sánchez-Lafuente, 2000; Carlo & Morales, 2008). At the

103 same time, clumped distribution of fruiting trees might decrease seed dispersal distances because

104 frugivores move short distances to forage on different maternal trees (Carlo & Morales, 2008). This

105 contributes to the admixture of maternal progenies at the local scale that might maintain high

106 genetic diversity among deposition sites within a population (García et al., 2009; Giombini et al.,

107 2017). Therefore, the structure of forest canopy and particularly the density of fruiting conspecifics

108 might enhance or dilute the impact of frugivore loss on the distribution of maternal progenies in the

109 seed rain (Côrtes & Uriarte, 2013).

110

111 The extirpation of large-bodied avian frugivores has been previously associated with

112 microevolutionary shifts in seed size (Galetti et al., 2013; Carvalho et al., accepted) and allele

113 frequencies (Carvalho, Galetti, Colevatti, & Jordano, 2016) in our study species, the neotropical

114 palm *Euterpe edulis*. Here we characterize the spatial distribution of maternal progenies in a

115   frugivore-generated seed rain of the palm *Euterpe edulis* across areas subjected to different levels of

116   frugivore loss. By combining molecular tools with a thorough sampling of the frugivore-generated

117   seed rain we quantified the number of seeds, the richness of maternal palms contributing to the seed

118   rain (i.e., maternal progenies) and the number of maternal effective alleles in pre-established

119   deposition sites (Fig. 1). We analyzed these variables as a function of defaunation, density of

120   conspecific fruiting palms and forest canopy structure. Small to medium-sized birds, mostly

121   thrushes (*Turdus* spp.), visit palms more often than large birds and tend to remove fruits from

122   multiple fruiting trees. We expect that the richness of maternal progenies will be negatively

123   associated with defaunation because the lack of large frugivores hinder the input of new genotypes

124   from beyond the immediate neighborhood (Table 1). Because large frugivores are usually more

125   mobile than medium-sized frugivores and disperse seeds to distant locations (Bueno et al., 2013;

126   Holbrook & Loiselle, 2009), we also expect seed dispersal to be more spatially limited in

127   defaunated areas (Pérez-Méndez et al., 2016), so that deposition sites are less likely to receive

128   dispersed seeds. Also, we hypothesize that the richness of maternal progenies will reflect the local

129   density of conspecific palms owing to the intense and local foraging activity of *Turdus* spp. (Table

130   1). Finally, we hypothesize that the amount of overlap in the composition of maternal progenies

131   between deposition sites will be higher in forests with richer avian assemblages due to frugivores

132   that act as mobile links among patches in the forest (Table 1). This is because large avian birds tend

133   to ingest many fruits at once and can move long distances, promoting dispersal of siblings across

134   multiple sites.

135

136   **Material and Methods**

137

138   *Study area and species*

139   The Brazilian Atlantic Forest is an ideal system to test the effects of anthropogenic

140   defaunation on ecological and evolutionary processes because it has been intensively disturbed by

141   human activities for centuries; however, we can still find preserved areas along its distribution. The

142   Atlantic Forest originally covered the Atlantic coast of Brazil extending from the Northeast to the

143   Southern region of the continent, including parts of Argentina and Paraguay (Joly, Metzger, &

144   Tabarelli, 2014). Today it has been reduced to 12% of its original extension, which is estimated in

145 150 million ha (Ribeiro, Metzger, Martensen, Ponzoni, & Hirota, 2009). Habitat fragmentation,
146 among other drivers, has caused a continuous and alarming loss of biodiversity during the last ~500
147 yr, resulting in a landscape composed of natural habitat islands surrounded by croplands, pastures
148 and urban matrix (Joly et al., 2014). The juçara palm (*Euterpe edulis,* Mart. Arecaceae) was once
149 one of the dominant trees in the Atlantic forest but it is currently endangered and locally extinct in
150 many areas owing to illegal harvesting of its edible meristem (Galetti & Fernandez, 1998). *Euterpe*
151 *edulis* is a self-compatible monoecious species with predominantly outcrossed reproduction
152 (Gaiotto, Grattapaglia, & Vencovsky, 2003) pollinated by small-sized bees (e.g., *Trigona spinipe*s).
153 This species bears fruits once a year with the fruiting season usually spanning around three months
154 (Castro, Galetti, & Morellato, 2007; Galetti, Zipparo, & Morellato, 1999). This palm produces
155 ellipsoid fleshy fruits ranging from 6.7 to 16.6 mm in diameter (Galetti et al., 2013; Pizo, Von
156 Allmen, & Morellato, 2006), eaten by more than 58 birds and 20 mammalian species (Galetti et al.,
157 2013). Yet, seeds are mostly dispersed by a reduced subset of large frugivorous birds such as
158 cotingas (*Procnias nudicollis*), toucans (*Ramphastos* spp), and guans (*Penelope* spp. and *Aburria*
159 *jacutinga*) and medium-sized avian frugivores (*Turdus* spp.) (Bello et al., 2017; Galetti et al., 2013).
160 Historically, our study areas shared a similar assemblage of seed dispersers, but forest
161 fragmentation and hunting have impoverished the assemblage of large vertebrates leading to
162 frugivore downsizing across the Atlantic Forest (Galetti et al., 2013). There is no data available on
163 when defaunation happened in our study areas, but it is known that deforestation in this region dates
164 back to the 1800s, through the conversion of continuous forest into agricultural field (mainly
165 coffee) (Dean, 1976 apud Galetti et al., 2013).

166

167 *Sampling design*

168   We selected ten study areas in the southeastern region of the Brazilian Atlantic Forest (Fig.
169 2, Supporting Information Table S1). In each area we set and georeferenced (with a Garmin GPS)
170 15 deposition sites (Fig. S1) that consisted of two seed traps of 1×1 m placed side by side and
171 suspended 1 m above the ground. The deposition sites were distributed along transects in each study
172 area. We assured that each deposition site was placed at least 50 m apart from each other and not
173 directly below a fruiting palm to prevent collection of fruits not dispersed by frugivores. Density of
174 fruiting palms in the immediate vicinities (within 8m-radius) of the deposition sites varied widely in
175 all study areas (from 0 to 26 fruiting palms per vicinities). Seed traps were monitored and dispersed

176 seeds collected every 40 days during the fruiting season of *E. edulis*, which usually spans around 3
177 months from April to September depending on the area (Galetti et al., 1999). Seeds were sampled
178 from four consecutives fruiting seasons (years 2013-2016). Dispersed seeds were collected and
179 stored in a paper bag until further analysis in the laboratory.
180
181   To characterize the local environmental heterogeneity of the forest canopy we set an 8 m-
182 radius centered in between each pair of seed traps (i.e., deposition site) to measure two sets of
183 variables. This radius was chosen because a study using eleven 25x50 m plots from six areas along
184 the Atlantic Forest found that most *E. edulis* seedlings are within 8 m of the nearest adult palm
185 (adult stems), which may be evidence that the bulk of seed dispersal events happens within this
186 interval (Valverde et al. in review). The first set of variables characterizes the conspecific
187 neighborhood, including: (i) the number of fruiting palms; and (ii) the distance to the nearest
188 fruiting palm. These variables were evaluated once a year and, because they did not vary
189 considerably across years, we used the minimum distance to the nearest fruiting palm and the
190 maximum number of fruiting palm over the years. It was possible to identify all individuals that
191 fruited in each season because the empty infructescence remains attached to the palm for a long
192 period. The second set of variables depicts forest canopy structure in terms of: (i) distance to the
193 nearest gap opening; (ii) number of total trees with dbh > 10 cm; (iii) and dbh > 30cm; (iv)
194 percentage of open canopy above deposition sites; (v) leaf area index (LAI); and (vi) canopy height.
195 These variables were measured only once in the first year of sampling. We considered as "gap
196 opening" an area with partial or complete removal of tree crowns, leading to a higher incidence of
197 light. Percentage of open canopy and LAI were estimated from hemispherical photography with
198 Gap Light Analyzer – GLA (Frazer, Canham, & Lertzman, 1999). The hemispherical photographs
199 were taken with a Nikon Fisheye Converter FC-E8 (Nikon, Tokyo, Japan) and the camera was
200 placed at the center of each deposition site, 1.30 m above the ground and oriented so that the top of
201 each photograph pointed north. The number of fruiting palms and distance to the nearest fruiting
202 palm were highly correlated ($r = -0.62$, $p < 0.001$), thus we retained only the first variable in further
203 analysis. To depict forest structure, we applied a principal component analysis (PCA) to a matrix
204 containing all above-mentioned variables (columns) per deposition sites (rows). We retained the
205 first PCA axis (PCA1) that explained 40% of the total variance. Positive values of PCA1 represent
206 deposition sites with denser and higher forest canopy, while negative values indicate sites with open

207 canopy (Fig. S2). PCA analysis was performed with the *stats* package (v 3.4.0) implemented in R (v

208 3.4.0) (Team, 2014).

209

210 *Defaunation level*

211   We obtained the composition of the avian disperser community in each area based on

212 frugivory observations and census studies (Bello et al., 2017; Galetti et al., 2013). The defaunation

213 level (*D*) was measured as the difference between the number of species of seed dispersers in the

214 richest avian assemblage (Site A, n = 21, *D* = 0) and the number of species of seed dispersers found

215 at the focal area, with area J being the most defaunated (n= 4, *D*= 17) (Table 2, Fig. 2). Thus,

216 defaunation levels were based on presence-absence data only. Despite the loss of some large-sized

217 frugivore birds, areas A-C presented very similar defaunation levels ranging between 0 and 4. The

218 frugivore assemblages of these study areas were composed on average by 20 frugivores that

219 included families of large birds such as Ramphastidae, Trogonidae, Contigidae and Momotidae; and

220 medium-sized birds of the Turdidae family (Fig. 2 and Table S3). Study areas D-F presented

221 intermediate defaunation values. The most defaunated areas were study areas G-J, with frugivore

222 assemblages mainly including species of the Turdidae family (i.e. thrushes). However, this index is

223 still useful as it reflects the loss of large birds across the areas, enabling us to test our hypotheses.

224 Estimating species abundance for the entire frugivore assemblage in tropical areas is not an easy

225 task, because many species are rare, cryptic, and the vegetation structure makes it difficult to locate.

226

227 *DNA extraction and genotyping*

228   We identified the genotype of the mother tree for each dispersed seed (maternal progeny) by

229 genotyping its endocarp (i.e., maternal tissue) (Godoy & Jordano, 2001). We attempted to genotype

230 at least ten seeds from each deposition site, but we failed to obtain this number in some deposition

231 sites either because there were fewer seeds available or due to poor DNA amplification. Overall, we

232 extracted DNA from 1330 endocarps following the protocol described by Lucas et al. (2019). All

233 endocarps were genotyped using seven highly polymorphic microsatellite loci (EE3, EE23, EE25,

234 EE45, EE47, EE52 and EE54) (Gaiotto, Brondani, & Grattapaglia, 2001), following PCR protocol

235 described by Lucas et al. (2019). DNA fragments were sized in ABI Prism 3100 automated DNA

236 sequencer (Applied Biosystems, Foster City, CA, USA) using GeneScan Rox 500 size standard

237 (Applied Biosystems), and scored using GeneMapper v.4.1 software (Applied Biosystems). We

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

241

242　　　We recorded a high rate of amplification failure possibly due to DNA degradation,
243　contamination from fungal or microbial DNA or the presence of polymerase inhibitors, such as
244　humic acids. These recurring failures led to a high number of incomplete multilocus genotypes. To
245　avoid excluding incomplete multilocus genotypes we defined the minimum number of loci
246　necessary to distinguish individuals with high confidence (Supporting Information 4 – Fig. S4 and
247　Table S4). We used complete multilocus genotypes of 296 adult palms from five study areas (two
248　defaunated and three with richer frugivore assemblages) to infer the probability of identity as a
249　function of the number of loci and determine the minimum number of loci for which no two
250　individuals share the same genotypes (Waits, Luikart, & Taberlet, 2001). We found that a minimum
251　of six completely scored loci were sufficient to obtain distinct multilocus genotypes for the set of
252　296 adults. 646 endocarps out of 1330 (48 %) were successfully genotyped for at least six loci, and
253　all subsequent analyses were performed using the set of 646 endocarps (mean of 5.71 [3.75 SD]
254　seeds analyzed per deposition site). For these endocarps, all microsatellite loci showed
255　polymorphism ranging from 19 to 27 alleles (Table S4). We used Microchecker v 2.2.3 (Van
256　Oosterhout, Hutchinson, Wills, & Shipley, 2004) to search for genotyping errors and we used ML-
257　Null Freq (http://www.montana.edu/kalinowski/software/null-freq.html) to infer the frequency of
258　null alleles using maximum likelihood estimation (Kalinowski & Taper, 2006) that varied between
259　0.02 and 0.07 across loci (Table S4). The overall missing-data load of our final data set was 2.2%.

260

261　*Dispersal and allelic diversity analysis*

262　　　We identified maternal progenies (i.e., seeds from the same mother tree) by matching the
263　multilocus genotypes of dispersed endocarps using the R package *allelematch* v 2.5 (Galpern,
264　Manseau, Hettinga, Smith, & Wilson, 2012), allowing one allele mismatching to account for the
265　possibility of genotyping errors that are expected when genotyping low-quality DNA sources, such
266　as dispersed endocarp tissues (García et al., 2009; Lucas, Carvalho, Hypolito, & Côrtes, 2019).
267　Therefore, multilocus genotypes that shared at least 13 alleles out of 14 were considered identical

268 and were assigned to a single maternal source. We have not repeated a subset of individuals to
269 estimate the rate of genotyping error. However, we estimate that the mismatch at one allele
270 corresponds to the genotyping error of 11% estimated using a similar set of loci (J. Santos,
271 Varassin, Muschner, & Ovaskainen, 2018). To characterize the richness of maternal progenies
272 within and across deposition sites, we estimated the number of different maternal genotypes at the
273 deposition site and the mean at the study area levels, respectively. Moreover, we quantified the
274 amount of overlap in the maternal progenies between deposition sites (the number of mothers
275 contributing simultaneously to pairs of deposition sites). Finally, we used the software GenAlEx 6.5
276 (Peakall & Smouse, 2012) to estimate the effective number of maternal alleles within deposition
277 sites and the mean across sites at the study area level.

278

279 *Influence of fruiting palm density, forest structure and defaunation on seed deposition, maternal*
280 *progenies richness and genetic diversity patterns*

281   The influence of fruiting palm density, forest structure and defaunation on seed deposition
282 patterns and seed rain genetic diversity were evaluated at the level of deposition site (n = 150) and
283 study area (n = 10). We performed these analyses at two levels because the variables were
284 represented in different scales: whereas defaunation values are unique measurements per study area,
285 density of fruiting palms and forest structure characterize the microhabitat around deposition sites.
286 Three models were tested at the microhabitat level, as follows: *seed dispersal model*- the effect of
287 fruiting palm density and forest structure (PCA1) on the number of dispersed seeds; *maternal*
288 *progeny model*- the effect of fruiting palm density, forest structure (PCA1) and number of dispersed
289 seeds on the number of maternal genotypes (maternal progenies); and *allele dispersal model*- the
290 effect of fruiting palm density, forest structure and number of dispersed seeds on the number of
291 maternal effective alleles. We fitted full models using zero inflated models because of the high
292 proportion of zero values in all response variables. We used the maximum-likelihood method using
293 R package glmmTMB (Magnusson et al., 2017), with the study area treated as random effect and
294 with a single zero-inflation parameter applied to all observations (ziformula=~1). Seed, maternal
295 progeny and allele dispersal models were fitted using negative binomial, Poisson, and normal error
296 distributions, respectively. The negative binomial and Poisson error distributions were used because
297 number of seeds and number of maternal genotypes are discrete counting data. Because seed
298 dispersal models showed overdispersion, we used negative binomial error distribution. The normal

299   error distribution was used in allele dispersal models because the number of maternal effective
300   alleles is a non-discrete variable. At the study area level, we tested five models. Instead of using
301   data of individual deposition sites, we used the mean number of dispersed seeds, mean number of
302   maternal genotypes, and mean number of effective maternal alleles across deposition sites, the
303   proportion of deposition sites that received at least one seed per area, the density of fruiting palms
304   per area and the defaunation level per area. The models were constructed as follows: two *seed*
305   *dispersal models*- the effect of density of fruiting palms and defaunation level on the mean number
306   of dispersed seeds per area and on the proportion of deposition sites that received at least one seed
307   per area; one *maternal progeny model*- the effect of fruiting palm density, number of dispersed
308   seeds and defaunation level on the mean number of maternal genotypes per area; one *allele*
309   *dispersal model*- the effect of fruiting palm density, number of dispersed seeds, and defaunation
310   level on the mean number of effective alleles per area; and one *defaunation model*- the defaunation
311   level was modeled as a function of forest structure (average PCA1 across deposition sites) and
312   fruiting palm density. Forest structure was not included in the seed dispersal, maternal progeny and
313   allele dispersal models at the study area level because it was associated with defaunation level, as
314   indicated by the results of the defaunation model above. Except for the variable of proportion of
315   deposition sites that received at least one seed per area that was modeled with binomial distribution,
316   all study area models were fitted using linear models with normal error distributions and the
317   maximum-likelihood method.

318

319   Because the number of genotyped seeds used to quantify the number of maternal progenies
320   differed across deposition sites, we included the number of genotyped seeds as an *offset* parameter
321   in all models. An offset variable is one that is treated like a regression covariate whose parameter is
322   fixed to be 1.0. It is useful when the response variable is estimated from different sample sizes, as
323   was the case for the variable richness of maternal genotypes. Starting with the full model, we fitted
324   nested models with all possible combinations of the fixed effects using the *dredge* function from R
325   package MuMIn (Barton & Barton, 2018), and then compared all models using AICc. The set of
326   best models (ΔAICc $\leq$ 2) were compared to reduced models without each predictor variable using
327   likelihood ratio tests (LRT, $\alpha = 0.05$). Significance levels of LRT ($p < 0.05$) are highlighted with *
328   in the Table 3. Finally, the results of best models were plotted using the *effects* R package (Fox,
329   2003).

330

Results

331

332

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

334   The number of dispersed seeds varied across deposition sites from zero (23% of deposition

335   sites) to a maximum of 325 (Table 2 and Fig. S5a,b). Overall, 53% of the deposition sites received

336   between one and 10 seeds and 23% collected more than 11 seeds. The number of dispersed seeds

337   per deposition site was positively affected by local palm density, but was not influenced by the level

338   of defaunation nor forest structure (Table 3, Fig. 3A and Table S5a,b). We found, however, that

339   deposition sites in more defaunated areas were less likely to receive at least one seed (p-value for

340   LRT = 0.01) (Table 3, Fig. 3C and Table S5a,b), indicating that there are more unvisited patches in

341   areas with fewer disperser species, which suggests dispersal spatial limitation.

342

343   *Influence of fruiting palm density, forest structure and defaunation on maternal progenies richness*

344   *and genetic diversity patterns*

345   In total, we identified 390 mother trees (different maternal genotypes) contributing to the

346   sampled seed rain of 646 seeds (Table 2). The number of mother trees contributing to the seed rain

347   varied widely among deposition sites and across study areas (Table 2, Fig. S5b). The number of

348   different maternal genotypes across the deposition sites varied from 0 to 12 in all areas. We

349   observed that most of the contributing mother trees contributed with a single seed dispersal event

350   (68.2%), but some trees contributed with up to 12 seeds in areas with richer frugivore assemblage

351   (Fig. S5b). At the microhabitat level, the null model was among the plausible candidate models

352   indicating that the number of different maternal genotypes was not influenced by any of the tested

353   covariates (number of dispersed seeds, fruiting palm density and forest structure) (Table 3 and

354   Table S5b). At the study area, however, the mean number of maternal genotypes was positively

355   influenced by the level of defaunation (p-value for LRT = 0.01) and the fruiting palm density (p-

356   value for LRT = 0.001 and 0.0001) (Table 3, Fig. 3D,E and Table S5a,b). The level of defaunation

357   was present in only one model in combination with palm density. The number of effective alleles

358   was not influenced by any of the covariates at the study area level; however, it was influenced by

359 the number of dispersed seeds at the microhabitat level (p-value for LRT = 0.03) (Table 3, Fig.3B
360 and Table S5a,b). Finally, the defaunation level was negatively associated with the forest canopy
361 structure, with denser and higher forest canopy associated with higher richness of frugivores (p-
362 value for LRT = 0.0006) (Table 3, Fig. 3F and Table S5a,b).

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

367

368 **Discussion**

369

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

382

383 *Influence of defaunation on seed deposition patterns and maternal progenies richness in the seed*
384 *rain*

385   Richer assemblages of avian frugivores, including species of medium- and large-sized
386 frugivores, were more prone to occur in areas with denser and higher forest canopy. Similar results
387 have been found in other Atlantic forest areas showing that the diversity of forest-dependent birds

388 decreases with the simplification of vegetation complexity (Morante-Filho, Arroyo-Rodríguez,
389 Pessoa, Cazetta, & Faria, 2018). It is possible that higher vegetation complexity provides higher
390 availability of food resources (Morante-Filho et al., 2018) or that hunting pressure co-varies with
391 selective logging that simplifies the vegetation complexity.

392

393   Our results show that plant populations lacking large-sized dispersers still receive
394 quantitatively efficient dispersal services (i.e. quantity of dispersed seeds *sensus* Schupp et al.,
395 2002) when an active set of medium-sized frugivores is still in place. This pattern was found in
396 other studies that report medium-sized seeded plants being dispersed by extant small- and medium-
397 bodied frugivores in degraded habitats (Farwig, Schabo, & Albrecht, 2017; Pérez-Méndez et al.,
398 2015). Previous studies have attributed this result to competition release caused by the extirpation
399 of the large seed dispersers, which results in augmented densities of small- and medium-sized
400 frugivores (Farwig et al., 2017; Kurten, 2013). In our study, this result can be attributed to the fact
401 that medium-sized frugivores are, quantitively, the most effective *E. edulis* seed dispersers in
402 defaunated and pristine forest (Carvalho et al., 2019; Galetti et al., 2013; Rother, Pizo, & Jordano,
403 2016). Thus, the loss of large frugivores might not impact the amount of seed being dispersed. In
404 contrast to quantitative aspects, our results showed that the loss of large frugivores reduced some
405 qualitative aspects of dispersal services. Deposition sites in areas with impoverished fauna were less
406 likely to receive at least one seed, indicating that dispersal is more spatially limited. The spatial
407 dispersal limitation can preclude seeds to reach and colonize new microhabitats and, therefore,
408 intensify plant aggregation over time (Schupp et al., 2002).

409

410   The distribution of maternal progenies in the seed rain can be explained by differences in
411 foraging behavior between medium- and large-sized frugivores. Thrushes (*Turdus* spp.) are the
412 main seed dispersers of *Euterpe edulis* (Galetti et al., 2013; Rother et al., 2016) because they occur
413 in pristine and degraded habitats and are the species that remove the largest amount of seeds. Their
414 small body and gape sizes allow thrushes to consume and disperse only one or two seeds per visit
415 when they forage on *E. edulis* (Galetti et al., 2013; Rother et al., 2016). Their limited movement
416 patterns and fast gut retention time (Morales, García, Martínez, Rodriguez-Pérez, & Herrera, 2013)
417 result in local dispersal patterns (García et al., 2009; Jordano, 2017). Therefore, thrushes most likely
418 generate seed rains with high number of mother trees at the microhabitat and study area levels. In

419 contrast, large-sized frugivores, only found in more preserved areas, consume large quantities of
420 fruits per visit (Galetti et al., 2013; Rother et al., 2016) and, therefore, are more likely to disperse
421 numerous sibs propagules to the same deposition site (Jordano, 2017). Consequently, the average
422 number of maternal genotypes was not negatively impacted by defaunation. These results indicate
423 that medium-sized, abundant frugivores, are key to maintaining local genetic diversity in areas
424 subjected to different levels of human-driven perturbation. The mixture of maternal progenies
425 provided by these frugivores may be crucial to the local demographic cycle of *E. edulis* because
426 experimental studies have documented influences of the genetic relatedness among neighbor seeds
427 on germination and seedling survival rates (Browne & Karubian, 2016; Scofield et al., 2012).
428 Nevertheless, it is worth mentioning that, because of high seed size variation, small seeds of *E.*
429 *edulis* can still be dispersed in defaunated areas (Carvalho et al., accepted). It is important to note,
430 however, that different outcomes would be expected for larger seeded plant species that can only be
431 dispersed by larger bodied frugivores. In the Atlantic Forest, for example, species with seed length
432 > 16 mm represent about 28% of vertebrate-dispersed species (Bello et al., 2017). For these species,
433 medium-sized frugivores are not able to compensate for the loss of larger frugivores and we would
434 expect a decrease or collapse of seed dispersal at local and landscape scale (Pérez-Méndez et al.,
435 2016; Pérez-Méndez, Jordano, & Valido, 2018).

436

437   Overall, each deposition site contained seeds from a different set of mothers, suggesting
438 maternal progenies are highly spatially structured in all areas. We did find, however, a few events
439 of shared maternal progeny between deposition sites in areas with large-bodied frugivores, which
440 may indicate overlap in spatially ample seed shadows generated by these birds. These results
441 suggest that large frugivores are able to move and disperse seeds of *E. edulis* beyond the immediate
442 vicinities of maternal plant, promoting small scale gene flow between microsites. More defaunated
443 areas did not present any sharing of siblings between deposition sites, suggesting that defaunation
444 of large-bodied frugivores lead to the collapse of movements away from the genetic neighborhood
445 (Jordano, 2017). Thus, although medium-sized frugivores are able to maintain local genetic
446 diversity in fragmented and animal-impoverished landscapes (Carvalho et al., 2016), the loss of
447 large frugivores impose a dispersal limitation in distance for this species, which may have lasting
448 effects on gene flow and genetic structure (Carvalho et al., 2016; A. S. Santos, Cazetta, Dodonov,

449　Faria, & Gaiotto, 2016). It is undeniable, however, that we could have observed more instances of
450　shared progenies among deposition sites if sampling was to be continued.

451

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

460

461　*Fruiting palm density influence seed deposition patterns and maternal progenies richness in the*
462　*seed rain*

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

474

475　*Conclusion*

476　　　　We suggest that medium-sized frugivores disperse large amount of seeds and contribute to
477　the maintenance of high local genetic diversity in human degraded forests. Nevertheless, the loss of
478　large-sized seed dispersers impacted the quality of seed dispersal service, mainly in terms of

479 dispersal away from the local neighborhood, with anticipated negative effects in the long term for
480 the persistence of a key palm tropical tree *E. edulis*. Our results are consistent with previous
481 findings on the effect of defaunation on genetic diversity and differentiation of *E. edulis* populations
482 (Carvalho et al., 2016; Carvalho, Ribeiro, Côrtes, Galetti, & Collevatti, 2015; Carvalho et al., 2019).
483 Previously, we reported that the functional loss of large seed dispersers resulted in genetic
484 differentiation among *E. edulis* populations; however, unexpectedly, we did not find evidence that
485 defaunation would reduce genetic diversity or increased fine-spatial genetic structure at the
486 seedlings stages (Carvalho et al., 2016; Carvalho, Ribeiro, Cortes, Galetti, & Collevatti, 2015).
487 Because we lacked understanding about the fine-scale processes generating spatial and genetic
488 distribution of *E. edulis*, these patterns were attributed to time-lag effects between defaunation and
489 genetic changes. The present results indicate that the maintenance of genetic diversity in frugivore
490 impoverished areas may be driven by the dispersal activity of remnant medium-sized frugivores in
491 dispersing seeds of different mother trees to the same deposition site. Thus, our study highlights that
492 medium-sized frugivores contribute to ecological services to a threatened tropical palm and,
493 therefore, may be critical for promoting evolutionary endurance under scenarios of rapid
494 environmental change.

495

505

506 **Authors Contributions**: CSC and MCC conceptualized and performed the study design. CSC
507 collected, analyzed the data and wrote the manuscript. MSL helped collecting samples and

508 contributed to writing the manuscript. CG, MCC, and PJ contributed to data analyses and to writing
509 the manuscript.

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514

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Figure legends

700 **Figure 1:** Scheme illustrating the expected richness of maternal progenies in frugivore-generated
701 seed rains in forests with richer frugivore assemblages (A) and defaunated forest areas (B). Within-
702 deposition sites represents the richness of maternal progenies; and between-deposition sites
703 represents the overlap of maternal progenies between pair of deposition sites. Circles represent
704 dispersed seeds and colors indicate mother trees (i.e., seed sources). Therefore, circles of the same
705 color are progeny seeds from the same mother tree (i.e., siblings). Diagonally crossed circles
706 indicate seed deposition sites that do not share maternal progenies.
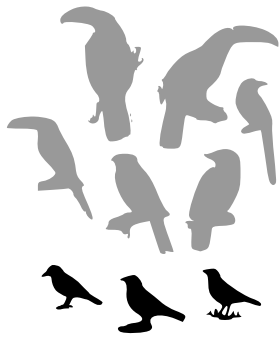
707

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

717

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

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

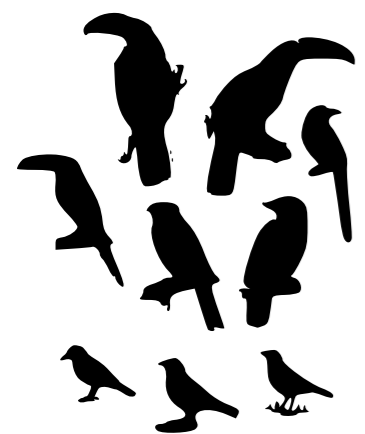|	Ecological theory	Expected effects on seed dispersal, maternal composition and genetic diversity in the seed rain
Greater defaunation 	Small birds are the most abundant in defaunated and non-defaunated areas. They ingest one or few seeds per visit but are frequent visitors. Defaunation may affect plant 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 trees 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	(+) Number of dispersed seeds (+) Richness of maternal progenies (+) Number of effective alleles

seeds from different sources.

728 **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

729 we report the defaunation level; fruiting palm density; mean number of sampled dispersed seeds per deposition site; percentage of the 15

730 deposition sites that did not receive any dispersed seeds; total number of genotyped endocarps; total number of maternal progenies per study

731 area; mean number of different maternal progenies per deposition sites in the study area. The names of study areas are in Table S1.

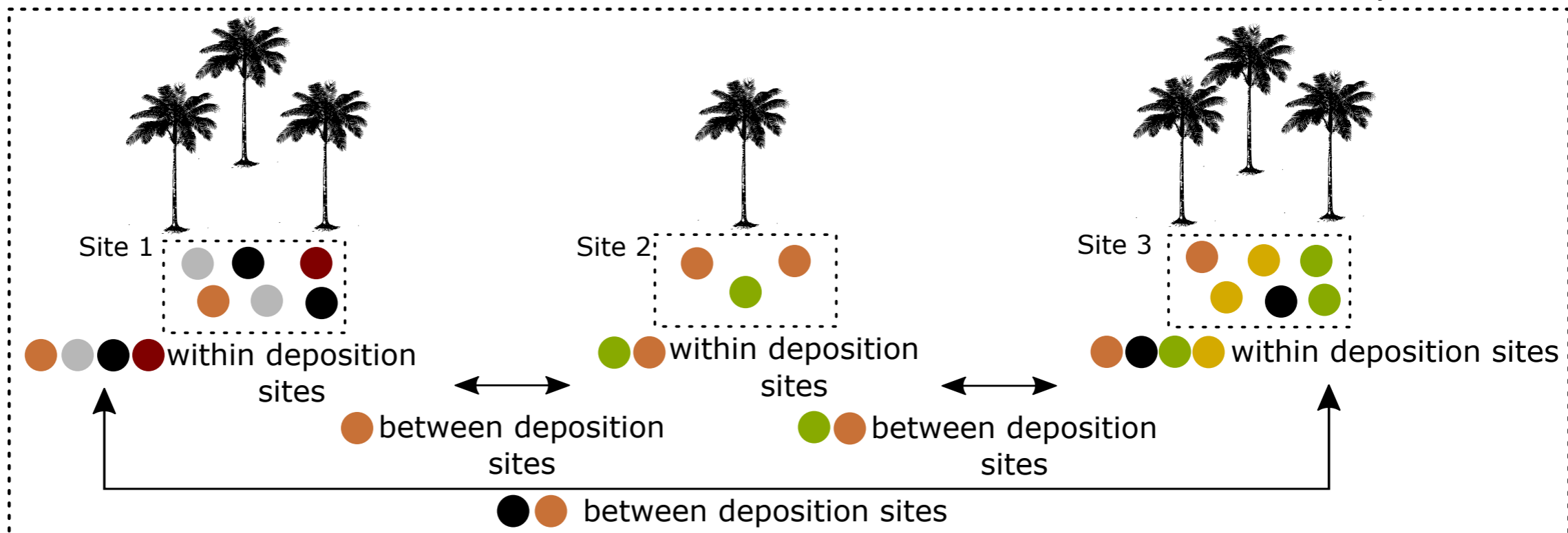| Study area	Defaunation level	Fruiting palm density	Mean N of dispersed seeds (SD)	% of empty deposition sites	N of genotyped endocarps	Total N of different maternal progenies	Mean N of different maternal progenies (SD)
A	0	8.33	13.00 (9.55)	0	98	52	3.46 (1.30)
B	1	0.80	2.75 (4.22)	33	57	27	1.80 (1.69)
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)

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 loglikelihood. Significance levels are highlighted with *$p < 0.05$. Information about these models is available in Table S5a in the Supporting Information.

	Response	Model[a]	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

743

Population A

Population B

Defaunation

Site 1

within deposition sites

between deposition sites

between deposition sites

Site 2

within deposition sites

between deposition sites

Site 3

within deposition sites

Site 4

within deposition sites

between deposition sites

between deposition sites

Site 5

within deposition sites

between deposition sites

Site 6

within deposition sites
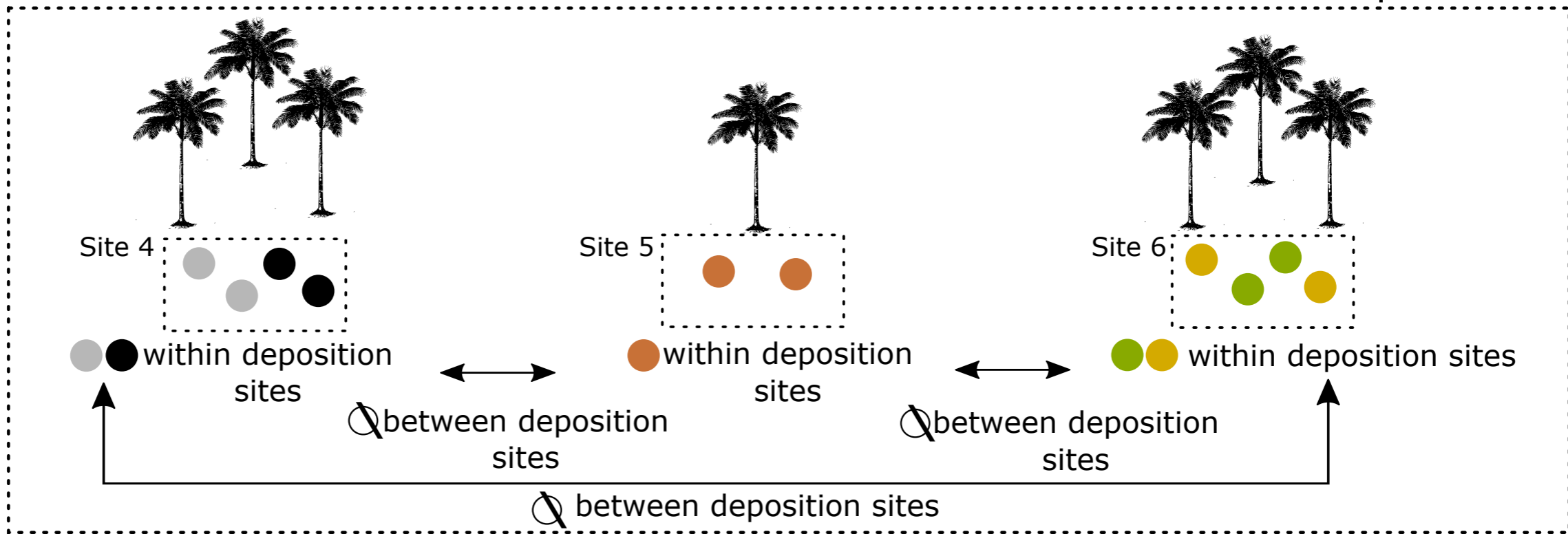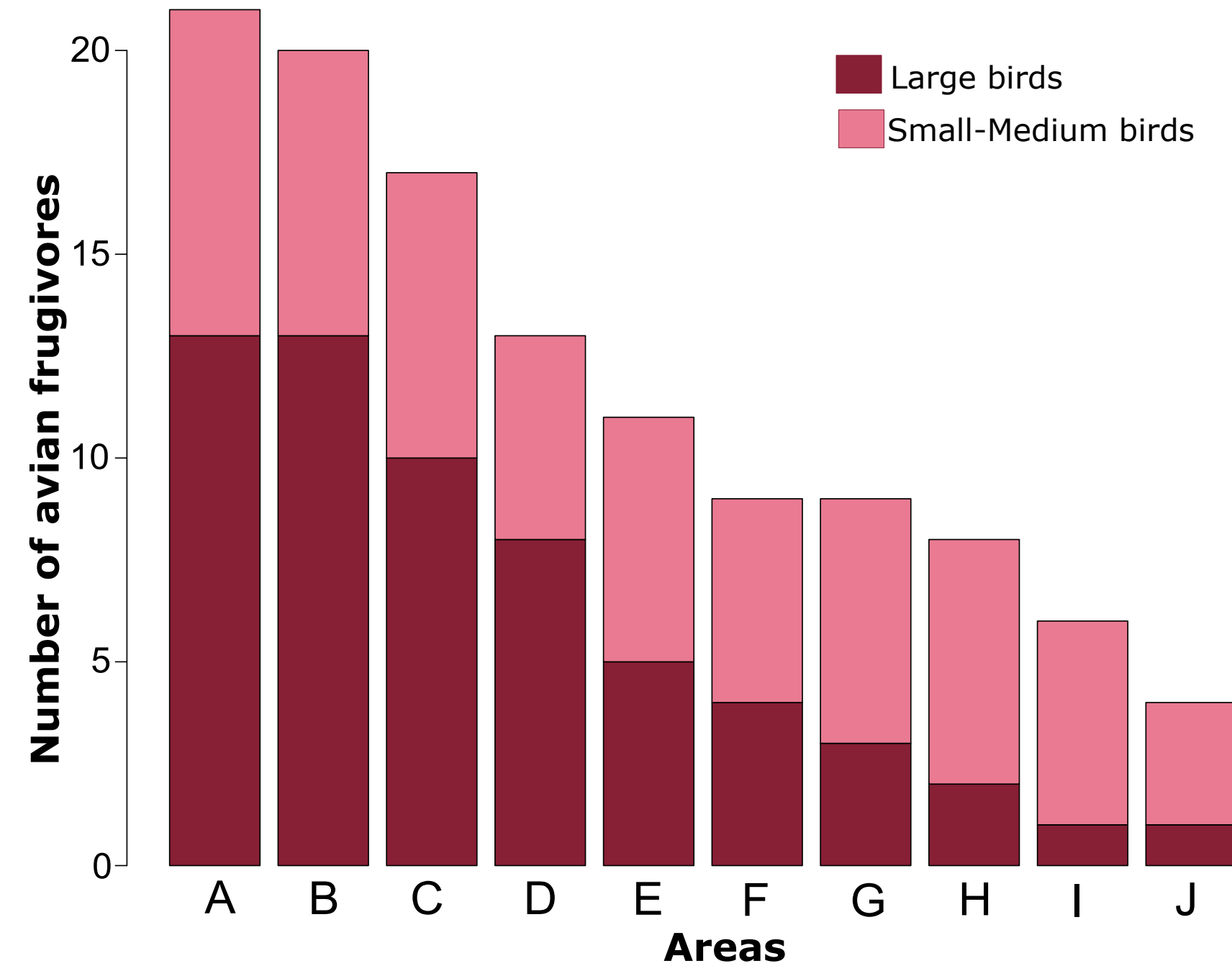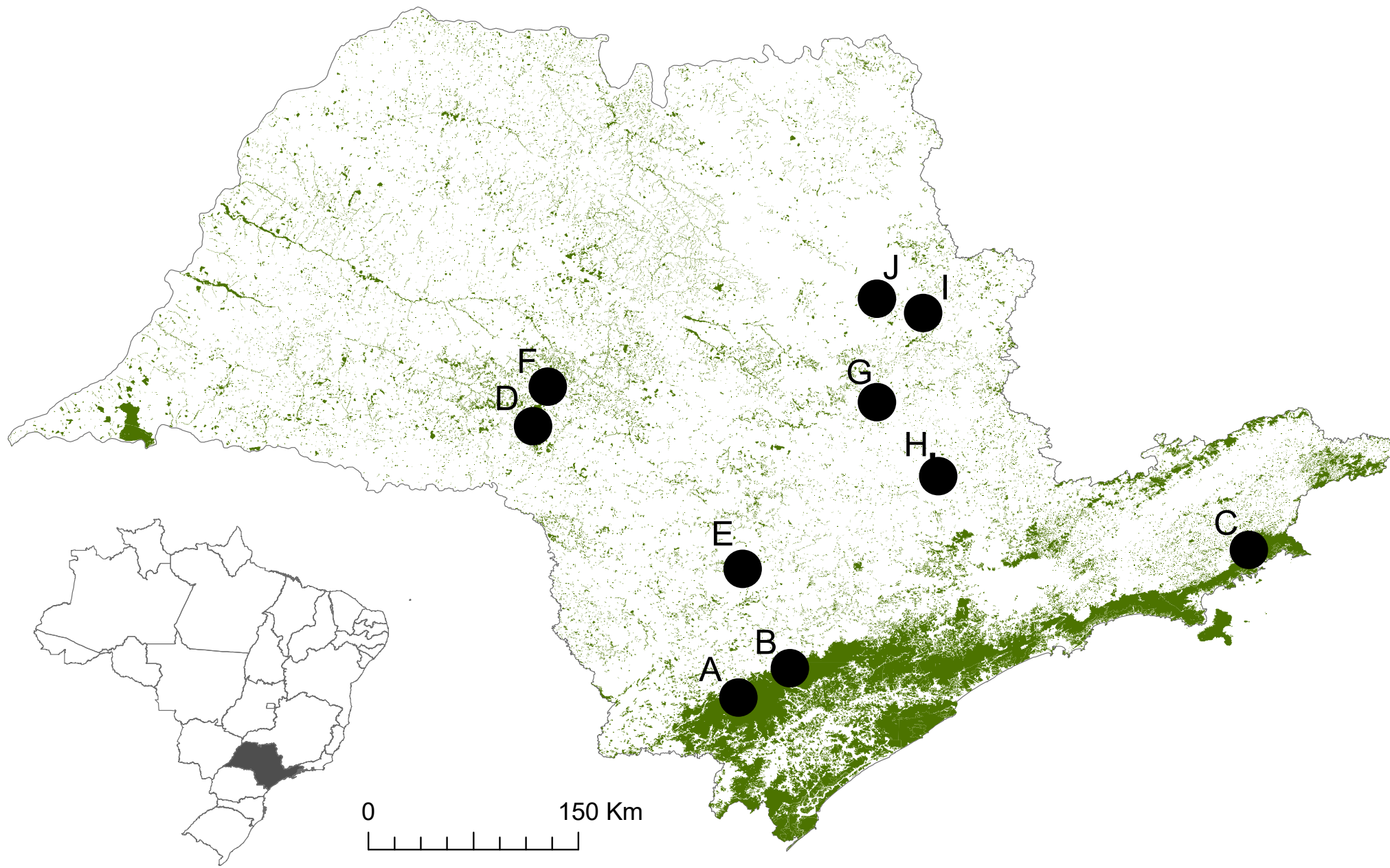
Microhabitat level

Study area level